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NOTES ON THE BEHAVIOR OF THE FIDDLER CRAB.

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There exists already a small literature dealing with habits of fiddler crabs and the general biology of these forms is known fairly well. As far as I am aware none of the various reactions of the animals, however, has yet been studied thoroughly. A *careful* study of the movements of an animal undoubtedly differs from a general one not merely in bringing some new details, but as well in bringing quite new problems. I quote Schwartz and Safir: "Correlated with the regular recurring changes in the tide, Uca performs its tasks with unchanging regularity, its general behavior never deviating from its standard, never altering from its established method, being almost stereotyped" (18 p. 20). "It has few tasks to accomplish, and does them day in and day out in the same way" (ibid.). From a certain point of view a crab has but a few tasks to accomplish, as digging the burrow, feeding, fighting and performing its sexual activities. From a similar point of view the "general behavior" of man is also a monotony of reactions, like dressing, undressing, eating, walking, talking and sleeping. However we do not consider the man as being a stereotyped automate because we know a lot about our own life, and we know that every one of those reactions may be performed in infinitely different ways. And yet a careful observation of the activities of a fiddler crab leads to very similar conclusions. Every individual digs its burrow but the details of this process may be infinitely various and the behavior of the animal is as far as possible from a stereotype.

My time being very limited I succeeded in observing but few

particulars. Nevertheless they seem to indicate that the existing literature on the fiddler crabs has not exhausted all possibilities.

The Burrowing Instinct.

All my observations were done on *Uca pugilator*, the sand-fiddler. In the environs of Woods Hole this species lives on sand banks communicating with the ocean by means of a complicated system of ponds and channels. On account of this circumstance the rising tide is very quiet and the animals are never exposed to waves. In fact so slow is the movement of the water that at the low tide one may see clearly sand-pellets removed by the animal from its burrow during the previous low tide. This particular is rather important to note, as many habits of *Uca* are correlated even with slow tidal changes.

Watching the animals in the field furnishes only data which are already known and there is no need to relate them once more. But one particular concerning the shape of the natural burrows deserves some attention. I studied it, pouring a solution of white plaster into the burrow and digging the mould out after it became hard. According to Pearse (12, 13) the burrows of *Uca pugilator* rarely exceed 75 cm., which I found to be correct. The burrows are nearly always oblique to the surface of the ground and they show a general tendency to become somewhat horizontal at the inner end. But there are very many modifications and it would be rather difficult to say which is the type. Sometimes the beginning of the burrow is vertical and then it bends sharply, as in Fig. 1, *a*; sometimes the inner end of an oblique burrow suddenly becomes vertical as in Fig. 1, *c*. They show, however, some common features, as they are always somewhat bent, never perfectly straight. Every burrow ends with a marked swelling—the end-chamber—where the animal often remains. In some cases the end-chamber is so large that its inhabitant may turn in it in every direction, in others it allows only turning around the transversal axis of the body. I failed to notice any difference connected with the sex of the crab. Also I have never seen a branching passage, as described by Cowles for *Ocypoda arenaria* (5). I found such a passage in some burrows of *Uca pugnax* but the burrow of *Uca pugilator* is

always a simple tube. It is difficult to say to what conditions may be due the marked individual differences in the shape of the burrows, as the external conditions on a horizontal bank of pure sand seem to be perfectly uniform for all individuals. Referring them to the different individuality of the crabs may be true, but probably it means nothing more than our complete ignorance of the causes.

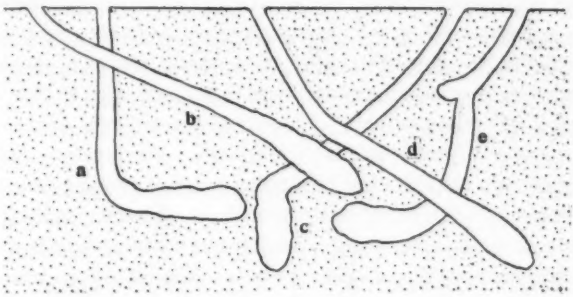

FIG. 1. Different types of natural burrows.

To be able to watch closely the process of burrowing we must observe it in the laboratory. In cylindric glass-jars (6 inches in diameter, 9 inches high) half filled with wet sand the crabs struggle madly and try to escape by climbing on the glass wall, in which they are of course unsuccessful. The animals remain near the wall and particularly near the most lighted spot of it. After some time the movements become slower, the crab gives up its attempts to escape and it keeps quiet for a while in its normal position. Finally it begins to dig. The individual behavior may be very different. Some crabs start their work after a few minutes, others roam around the jar for hours and even days. Some remain motionless for many hours, others move perpetually and struggle for escape. Some may walk around the wall showing no particular predilection for the lighted side, some again remain for a long time at the spot nearest to the window. There is a slight difference between the sexes, the females being more shy on the average and starting the work sooner. The mode of working is also somewhat different in both sexes, which will be mentioned later.

We come to our first question: on what conditions depends the spot at which the animal starts digging? The spot is not quite a random one as some rules seem to hold. Practically in all cases the hole lies close to the wall of the jar and at least very often the wall directed towards the light is preferred. The answer is a simple one. The crab is both positively phototactic and thigmotactic. During its struggling for escape *Uca* remains at the most lighted spot of the wall; there it quiets down and there it begins to dig. This explanation may be correct, but it leads to further considerations. Phototaxis means of course an overwhelming reaction towards the light, and only in this sense it has a definite meaning. We are also positively phototactic and we do not like to remain in the dark. Nevertheless nobody would explain the reactions of man on this basis as our phototaxis is checked by very many other reactions. Such an explanation, however true in some cases, would be one-sided and rather poor. But the same holds also for the crab. The animal is positively phototactic, and yet it digs a burrow which conducts it away from the light. During the burrowing *Uca* repeatedly comes out of the hole towards the light and it enters the burrow which is dark. Is then the phototaxis changing with every minute? In the jar the crab keeps usually close to the wall during the whole work and the whole burrow may be fully exposed to light, which does not disturb the animal in any way. After having finished the work and after having filled the burrow almost entirely with sand, as described below, the crab remains at the bottom of the jar, in the end-chamber, which is often fully lighted, and it may remain there motionless for days. We can compel the fiddler to dig in the middle of the jar making there a shallow hole and driving the crab into it. But as the burrow is oblique the animal soon reaches the wall of the jar at some particular spot and it makes an end-chamber there, being by no means disturbed by the light. We must consider that a similar situation never occurs under normal life conditions of *Uca* as the hole always leads to the dark. The natural burrows have their openings just as often directed to the south as to the north. All the evidences indicate very strongly that under fairly normal conditions of life *Uca* is insensible to light, or, to say it more correctly,

phototaxis does not play any marked rôle in its life. According to Schwartz and Safir on cloudy days the fiddlers remain inactive. On the contrary, in my laboratory hundreds of fiddlers displayed the most activity in the dark which may depend on being undisturbed when nobody remained in the room. The inactivity on cloudy days is surely connected with heat, not with the light. What we call phototaxis in *Uca* may be observed only under very unnatural conditions when the crab is struggling blindly for escape. Its activities are then uncontrolled by the inhibitory centers and automatic correlations may drive the animal towards the light. Such cases are very interesting for a study of automatic nervous connections but they are scarcely valuable for psychology.

As to the thigmotaxis some observations point to its existence. In the dark *Uca* also dig their holes near the wall but of course no particular spot of it is preferred to the others. And again a control experiment shows that the thing is not so simple. I put several crabs into a large crystalliser (11 inches in diameter) filled with sand. I thrust in the sand several small glass plates at different spots, covered the crystalliser and put the whole in the dark. If remaining close to the wall be only a matter of thigmotaxis we might expect that the crabs will dig just as often near the glass plates scattered through the whole surface of the sand, as they dig near the outer wall. But in fact from 26 crabs tested several times only one made the burrow near a peripheral glass plate, all others dug invariably close to the outer wall. And yet the sum of contact surfaces of all plates with the sand was even superior to the surface of the crystallizer's wall. Once more the thigmotaxis proves to be not an adequate explanation. The tendency of the crab is not to remain by the wall but to remain at the very periphery of the vessel. Under similar conditions practically all animals show the same behavior, *Paramœcium* as well as man. As I showed (6) *Paramœcium* swims along the periphery of the vessel in spite of its thigmotaxis being negative while swimming. In a closed place which we wish to leave we will be oftener found at the periphery than in the middle of it. The reaction has nothing to do with thigmotaxis.

Besides photo- and thigmotaxis there is a strong hydrotaxis, the crab striving towards the moisture. There exists also a very marked tendency of hiding itself in the sand. Especially in females we may see often a behavior distinctly different from the ordinary burrowing. It consists in a quick pressing of the body in the sand. As the friction is very strong the animal succeeds only in hiding the thorax while the legs of one side of the body remain uncovered. In this position the crab may remain motionless for hours and such a behavior certainly has some biological significance as very little is seen of the crab, the legs having a marked protective coloration. The consistency of the ground has also some influence on the choice of the spot where the work is started. If we make a shallow hole in the sand *Uca* will choose it as the starting point. All those "taxies" and strivings, and many more, determine the actual behavior and referring the activities of a crab to some particular tropism is rather a poor explanation.

However, at last the animal starts its work. To simplify the nomenclature I shall use roman numbers I.–V. for the legs of the side of the large claw of the male, and the arabic 1–5 for the legs of the opposite side. Thus I. means the large chela, 1— the small one.

At the very beginning of the work the side 1–5 is usually directed towards the wall of the jar, straightly or obliquely, and usually this side begins to dig. The legs 2, 3 and 4 which are

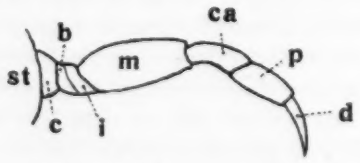

FIG. 2. The second leg from the side. *st*, sternum; *c*, coxopodit; *b*, basipodit; *i*, ischiopodit; *m*, meropodit; *ca*, carpopodit; *p*, propodit; *d*, dactylopodit. There is no movable articulation between *b* and *i*. Articulations *p–d*, *m–ca* and *c–b* work in the same plane, others at right angles to them.

bent in the articulations mero-carpopodit and dactylo-propodit at the normal position of the body become somewhat stiff. At the next moment they become bent a little more in both articu-

lations mentioned above and the terminal hooks (dactylopodits) sink into the sand. The legs are not bent at once but one after another in a quick rhythm which helps to make the sand lighter. If the sand is hard those efforts are repeated, until the whole dactylopodits and a good part of propodits enter into it. A portion of sand lies now between the legs 2, 3, 4 and the side of the thorax. Now the legs become also bent in the articulation coxo-basipodit which works in the same direction as the former two. The portion of sand becomes loosened from the ground and tightly pressed to the side of the carapace. The crab walks a few centimeters aside carrying the sand, then it stops and the legs 2, 3, 4 become bent also in the articulation sternum-coxopodit working nearly at right angles to the former three. Through this movement the pellet is directed towards the sagital plane of the body and forward. It is pushed in this direction by the side surface of the working legs. A further bending of the same articulation causes the legs to touch the pellet with their external surface. As soon as this occurred the legs become extended in the articulation mero-carpopodit one after another as at the beginning. The pellet may be now pushed still further towards the side I.–V. The sand is not very wet at the surface and the pellet does not hold together. The lose sand grains are to be prevented from falling back into the hollow remaining at the place of the removed pellet and the crab does it very carefully. In this work of pushing away the sand which is not any more held by the legs the small chela helps also. Very often it starts helping even before, when the pellet is first formed, pushing it together with legs 2, 3, 4. But it is never used for digging in the proper sense. The leg 5 also remains inactive in digging as it has a different task to accomplish. After having pushed the sand away, removed the grains that remained on the legs and kneaded the sand a little the animal returns to the hollow and grasps another portion of sand in the same way. But even the second pellet brings some difficulty with it as it is never carried far away and the lose grains of sand easily roll back. Therefore as the work progresses, more and more legs participate in it. Besides the legs 1, 2, 3, 4 the leg II. begins to help. When the pellet is already pushed beyond the sagittal plane of the body

the leg II. strongly bent in the articulation mero-carpopodit comes behind the pellet touching it with the external surface of pro- and dactylopodit. Extending the leg II. in the mentioned articulation the crab pushes and kneads the sand in the same way as do the legs 1–4. A little later also the legs III. and IV. are used in the same manner. Then the large chela begins to play its part. Its lower edge is held closely to the surface of the ground and extending the leg in the articulation mero-carpopodit *Uca* pushes from time to time large portions of sand away as with a spade. Many authors (Alcock, Pearse) consider the large claw of *Uca* as being a secondary sexual character. But it plays also an important rôle in digging.

The deeper the burrow becomes the higher is the sand hill at its mouth and the greater is the danger that the sand will roll back. All legs, excepting V. and 5, participate in pushing it away and this work is done more and more carefully. At the same time the sand from the deeper layers is more wet and the grains hold together better which facilitates the task. The pellets may be simply rolled away from the mouth of the burrow. In this action again all legs participate and the digging side presents the pellet to the pushing side which rolls it farther. The legs II., III., IV. push the pellet with the external side of their pro- and dactylopodits, the crab walking after it sometimes at a considerable distance. In the field the removed pellets often lie as far as one meter from the mouth of the hole. Sometimes when the chela I. has pushed a pellet far away it will do the same with some other pellets before the animal returns in the hole, as if the crab wanted to mend its former inaccurate work. The more sand is carried out the farther the crab rolls the pellet; sometimes it is deposited at the opposite side of the jar. The large chela, as mentioned, is used only from time to time; it does a rough work. In most cases it would only disturb the rolling of the pellets. Therefore it is always raised high in the air in the moment when the side 1–4 presents the pellet to the side II.–IV.

There is still the problem of locomotion to be solved. As known, during the sidewalk of crabs the legs directed forward pull the body while the opposite legs push it. As the digging

work progresses the crab has to carry its burden from a greater and greater depth. The legs II.–V. are free in pulling the body, but the legs 2–4 are holding the sand. The chief rôle in the locomotion on the inner side is played by the leg 5 which is never used for burrowing. In the later stages of burrowing the leg 2 also begins to help a little. Its articulations pro-dactylopodit and mero-carpopodit are moving and pushing the body, while the meropodit holds the pellet. To a certain extent also the small chela helps. Only the legs 3 and 4 remain motionless, holding the sand.

Another interesting particular. When the burrow is just deep enough to hide the thorax but the legs I.–V. remain outside, the crab often grasps the edge of the hole with legs II.–V. helping to move the body out of the burrow. There is the danger that the edge will break under this effort and the sand will fall into the burrow. Thus the crab grasps the edge at four points possibly distant from one another embracing about 160° of its circumference. In all other cases the legs are held much closer together.

The burrow is perfectly circular in every transverse section. This becomes possible because the crab changes very often its position within the tube turning around the transversal axis of the body. As the canal of the burrow is oblique the animal sometimes walks along it having its legs directed towards the upper wall and the back touching the lower one. Consequently *Uca* comes out from the burrow touching different points of the hole and this causes the pellets to be deposited at different spots around it. But the most of them are deposited in the direction which is a prolongation of the inclined burrow. Evidently walking along the lower wall of the canal is easier and occurs oftener.

Still repeating all those described movements the animal succeeds in digging a deep burrow ending blindly at the bottom of the jar. However sometimes *Uca* stops digging before the bottom is reached. As mentioned above the inner end of the burrow forms a kind of chamber markedly larger in diameter as compared with the canal. The animal is especially careful in making it and often it will spend hours in finishing the end-

chamber. The principal instrument is now the small claw which is repeatedly pressed with its external surface against the wall flattening the sand and hardening it. During the work the crab changes its position and it is often to be seen lying on its back and working at the roof. When the burrow is finished the animal will remain motionless, sitting in the end-chamber for a long time.

The female works in a closely similar way. But as both chelæ are equal there is no preference to either side of the body. In pushing the sand the claw of the side external to the burrow works as the other legs. From time to time the female will change the working side. On the average the work is accomplished a little sooner than in the male which is possibly due to the changing of the side digging which gives to it a chance for resting. One observation points in fact that the legs may soon become tired. When a crab runs along the sand for some paces it will stop from time to time quickly changing the side directed forward.

If the crab meets any obstacle while digging, its behavior depends very much on the stage of the work at which this occurred. Small stones are simply carried out like a sand pellet. Bigger stones which are too heavy for the animal cause an abandoning of the hole if they lay near the surface. In such cases the animal will start digging another burrow at some spot near to the first, changing the spot many times until it finds a suitable one. But if the obstacle lies deeper the efforts of the crab may last for a long time and often it rather prefers to change the direction of the burrow than to abandon a work which is half done. This has been observed already by Schwartz and Safir. The peculiar form of the burrow on the Fig. 1, *e*, is due to changing the direction of burrowing on account of some obstacle.

The described typical mode of working is connected with many difficult problems.

1. There is the characteristic shape of the burrow to be explained. If we make a perpendicular hole in the sand about 5 cm. deep and drive the crab into it the animal soon starts working. But it never goes in the direction of the hole. The

burrow becomes bent and it approaches continually to the horizontal plane. This tendency explains to a certain extent why the burrow is never straight. The animal must hide itself in the sand and the shortest way of doing it would be the digging in a vertical direction. On the other hand the burrow must approach the horizontal line. Both tendencies working together cause the burrow to become bent. From a mechanical point of view we might expect that the crab will dig in the direction of a diagonal. Thus the burrow forming a curve shows that the causes are more numerous and a mechanical explanation is far too simple.

The biological significance of the end-chamber and of the burrow being bent follows from a simple experiment. When the animal has finished its work and it is sitting motionless in the end-chamber we begin to drop slowly sea water into the jar. Very soon the water reaches the crab. The animal begins to stir, then it climbs up to the mouth of the burrow and closes it. To do this *Uca*, usually sitting in the burrow with its large chela directed towards the entrance, goes entirely out, turns and enters the burrow with the chela I. forward. When the body is so deep that the bent legs 2–5 touch the edge of the hole they grasp this edge and pull it strongly inward. This time the legs are held close together and the leg 5 participates in the work like the others. There results a pellet of sand which is dragged down and adjusted carefully to the side wall of the canal close to its entrance. The pellet is followed by the second, third, etc., until a solid cork is formed closing tightly the hole. On the surface of the sand at the spot where the hole was, there remain radial furrows converging towards the former center of the hole. This process was already described by Pearse and others. But the task is not yet finished. *Uca* grasps the sand inside the burrow and carries it to the top, adjusting the pellet to the new formed roof of the hole. Very many pellets may be handled in the same way and a thick cork arises which closes the burrow tightly. We may pour so much water as to cover the sand completely. If at the beginning we dropped the water in very slowly the animal had enough time to close the burrow carefully. In this case, and such is the case under natural

conditions, even under the water there remains always a waste chamber where air is retained and in which the crab remains until the tide goes out. This process may become modified in several ways. Very often *Uca* adjusts the sandcork in the middle of the canal without rising to the mouth of it. The end result remains the same, as the cork is also very tight. If we pour the water a little quicker the crab works in a great hurry. It rises to the top and it simply pulls many pellets of sand down without adjusting them. Sometimes the animal has no time to turn its large claw inward and it works hastily with II.–V. legs as well as it goes. Finally, if we pour the water very quickly, the burrow remains open, water enters into it, and the walls of the canal collapse burrying the animal. In such a case *Uca* usually very soon digs itself out and remains on the surface of the sand until we suck the water out with a pipette. In the field such an accident may be caused by a rain shower.

These observations pour some light on the biological significance of the burrow being bent and the end of it approaching the horizontal line. In such a burrow the end-chamber may be easier preserved from being filled with water. The air-chamber sometimes may be vertical as in the Fig. 1, *c*. But the foregoing part of the burrow is then strongly bent and the end result remains the same. Such air-chambers are known for several sand crabs, as for *Dotilla* (Symons, 19) and others.

Exactly speaking, the detecting of the biological significance of the shape of a burrow does not tell us anything about the factors that cause it. Beyond any doubt the direction of burrowing is closely connected with gravity. This follows from some experiments. In order to be able to watch closely the process of burrowing I constructed a simple apparatus. Two glass plates fitting closely into a jar in vertical position and reaching about two-thirds of its height were put into the vessel. Four corks of a suitable size (about 2 cm. thick) were put at four corners between the plates and the whole was held together with four threads. Thus the plates formed a kind of box and the 2 cm. wide space between them was filled with wet sand. Over this box standing vertically in the jar I put a round piece of cardboard fitting exactly to the jar and having a cleft in the

middle corresponding to the space between the plates. If we put a crab into the apparatus it begins to dig and as it can dig only between the plates it may be watched during the whole work. When the crab reached about the half of the height of the sand column I turned the apparatus at right angles. *Uca* changed then the direction of digging, working now nearly at right angles to the previous direction. Some of such burrows may be seen in Fig. 3.

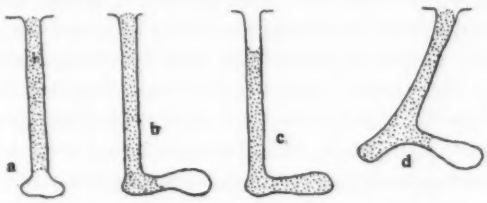

FIG. 3. Effect of turning the apparatus at 90° *a*, *b*, *c*, burrow of the same individual. For explanation see the text.

Clark (4) extirpated both inner antenna of *Uca pugilator*. He states that the normal equilibrium of the animal was somewhat damaged and there was a tendency to roll over. But at the same time the normal conditions, as feeding and burrowing, remained unaltered. Clark has not investigated the shape of the resulting burrows which could possibly furnish some interesting conclusions. For the moment we can say only that gravity is one of the factors affecting the shape of the burrow.

2. Another question connected with burrowing is the question of breathing. The interesting paper of Abbott dealing with the effect of distilled water upon the fiddler crabs (1) contains many important data. The gills of *Uca* are situated at the bottom of the large gill-chamber and the space over them is filled with liquid. Abbott thinks that this liquid has only about one-fifth of the concentration of the sea water. The gill-chamber communicates with the exterior by means of a canal, the opening of which lies between the basal joints of third and fourth legs. In this way the liquid of the chambers may exchange gas with the exterior. "In other words the crab when out of the water is able to breathe air" (1, p. 170). The invariable presence of an

air-chamber at the bottom of the burrow seems to support this view. It is probable in fact that the crab under its natural conditions practically is never out of the air. I kept several fiddlers in a jar the bottom of which was covered with a very thin layer of moist sand (2–3 mm.). The jar was covered and thus the atmosphere within it was saturated with vapor. The gill-chamber was prevented from drying but at the same time there was no chance of changing its liquid. Under such conditions the crabs lived very well for over 6 weeks and would probably have lived much longer had I not broken the experiment up. I failed to find anywhere data as to how long a fiddler may live under the water. Schwartz and Safir mention only that probably the fiddlers "do not find prolonged submergence very comfortable" (15, p. 19). And yet my fiddlers lived under the water for 6 weeks without showing any abnormalities and are still alive. To the end of this time they move rapidly when frightened and feed on mussels under the water. It is clearly to be seen that the flagella make their rhythmic movements, which in all crabs serve for renewing water in the gill-chamber. So the fiddler crabs seem to be true water-breathing forms like all other crabs. But at the same time the contents of their gill-chamber may be thoroughly ventilated in the air. It does not mean that they breathe air, nevertheless such a wide scale of adaptation furnishes an interesting example. The last experiment does not support the opinion of Abbott that the liquid of the gill-chambers has a lower osmotic pressure as compared with the sea water. It seems to me rather that this liquid cannot be anything else than the pure sea water.

3. A further question arises: why is the burrow of a fairly definite length, or, what conditions cause the crab to stop its working? There are several possibilities which may be tested experimentally.

The crab may stop after having expended a certain amount of energy in digging. But the resistance of the ground may be very different depending on the degree of moisture, presence of mud, small stones, plant roots, etc. The amount of spent energy may be very different in every single case and yet there is no evidence that the length of the burrow is affected by those

factors. They bear undoubtedly only on the time of digging. In some experiments I put a glass ring at the periphery of the jar covering it with a layer of sand about 3 cm. high. The crab digging close by the wall soon reached the obstacle and for several minutes it tried to remove it. It abandoned then the burrow and began to dig another one in the neighborhood which it abandoned in turn. The animal made 5 successive holes until it succeeded in bending the canal towards the middle of the jar and in avoiding the obstacle. The total amount of expended energy was certainly much greater than in the usual case and yet the animal dug its hole to the very bottom of the jar. The whole process lasted about 6 hours, during which *Uca* was constantly working, while an ordinary burrow is made easily in half an hour. In another series of experiments I mixed the sand with at least the same quantity of small stones (about 6 mm. in diameter). The resistance of the ground was very much increased, in fact about 3 times as measured roughly by determining the weight which was necessary for driving a nail into the sand and into the mixture. Nevertheless the crab succeeded in making a regular hole reaching to the bottom of the jar, which lasted however for about 20 hours. The amount of energy is different depending on the degree of inclination of the canal towards the horizon, as carrying the sand out from a steep burrow requires a greater strain. And again this does not bear on the length of the burrows.

Another possibility is that the crab may estimate the total length of the burrow while carrying the sand pellets out. But one observation speaks against the validity of this factor. The mode of burrowing described above is a type which does not mean however that it is to be observed the most frequently. In very many cases the crab works in a slightly different manner. When the burrow is just deep enough to hide the animal, *Uca* comes out of it, turns its big claw towards the burrow and enters again. The legs II., III. and IV. grasp the sand from the bottom, but the pellet is not carried out of the hole. The legs push it towards the opening, while the body remains at the same spot. The pellet comes to lie between the sternum of the crab and the wall of the canal. Still pushing it with the external

surface of the legs II., III., IV., the crab forces its own body below the pellet, grasping it with the legs 2, 3, 4. The sand is carried then to the mouth of the burrow and the legs 2, 3, 4, helped effectively by the small claw adjust the pellet at the edge of the hole. The second or the third pellet close the hole entirely, while the crab still continues the same work, grasping the sand at the bottom and adjusting it at the roof. As the work progresses the chamber containing the fiddler comes to lie still deeper under the surface and the sand-cork becomes still thicker. When the bottom of the jar is reached *Uca* spends a considerable time in working at the end-chamber and finally it quiets down, sometimes for many days. During the whole work the crab has no chance of measuring the length of the burrow as it is working itself through the sand. I think therefore that this factor may be also excluded.

If the importance of both mentioned factors is somewhat doubtful, it is sure that the degree of moisture plays a rôle in estimating the depth of the burrow. Even during the low tide I always found water at about one foot below the surface of the fiddler-ground. The burrow cannot go so far, for the walls of the end-chamber would collapse under the water. Testing the natural burrows with a long pipette I never found water there. The question may be solved experimentally. Usually I performed my experiments in the following way. I put sand into the jar and poured enough water to cover it completely. By stirring, droplets of air were removed. Then a long pipette was forced through the sand to the very bottom of the jar and the water was sucked out as far as possible. Under such conditions the crabs dug always to the bottom. But if we put the pipette only to half of the thickness of sand sucking water out, the animal will stop working when the level of water is reached. The degree of moisture influences then the depth of the burrow.

Unfortunately it is also sure that this factor is not the sole one. The natural burrows measured on an area of about one square meter on a perfectly flat ground may reach a very different level below the surface of the sand in spite of the exactly equal rising of the degree of moisture for all of them. Thus I am unable to answer exactly even such a simple question as why the burrow

is of a definite length. One is sure that the behavior of a *Uca* is determined by very many different factors.

4. The question of periodicity in the life of the fiddler crab is a very complicated one, as there are some theoretical objections. In my opinion the problem of intrinsic periodicity of the life phenomena resembles very much the famous problem of the inheritance of acquired characters. The rising tide brings a variety of factors with it and so does the falling tide. The life of a crab is fairly periodic and this is due to an approximative periodicity of external factors. Now if we observe the animal in the laboratory where conditions are fairly constant, there are no factors which in nature cause the rhythmicity of behavior. How could we expect such a rhythmicity? Every kind of activity is a reaction to certain stimuli. The peculiar character of all these reactions is hereditary and fairly constant for each species. But it surely does not mean that in the entire absence of those stimuli the reactions would remain the same. Such a "memory" is an obvious impossibility. If the causes are absent the effect will be absent also. We do not think that under natural conditions the periodicity of behavior of a fiddler is due entirely to the "memory" and not to the periodicity of tides. But only in this case we could expect the animal to behave rhythmically under the constant laboratory conditions. As to the cause of all rhythmicity, the tides, it is interesting to note that they are not rhythmic at all. I quote a random instance from Eldridge's Tide-Book for 1925. Successive intervals between the high-tide, low-tide, high-tide, etc., during the ten days are, in minutes:

349	308	389	316
406	380	360	426
341	356	324	364
401	399	419	376
346	312	385	311
410	430	360	431
353	381	321	349
389	371	419	390
344	321	379	308
412	421	365	434

The lowest interval is 308, the highest 434, which corresponds or a difference of 2 hours 6 minutes. As such an irregularity tuns all the year round it would be a pure wonder if the "memory" would compel an animal living under constant conditions to accomplish its tasks at the same intervals. I do not intend to discuss the rich literature on periodicity of organic functions, but I have a strong impression that the whole question has a lot of metaphysics in it.

In fact I never noticed any intrinsic periodicity in my crabs. When put into the jar the crab digs the burrow, closes it and remains in the air-chamber for a very various time. Sometimes it will dig itself out in a few hours, sometimes it remains quiet for 3–4 days. If we put water into the jar while the chamber is tightly closed the crab does not go out even for a week. If we suck all water out *Uca* usually digs itself out in 3–4 hours, but sometimes it does not stir for many days. Its behavior must be ascribed to actual external factors, not to the remembered ones, although we are very far from understanding which factors are working. When the sand begins to dry *Uca* closes the burrow. It does the same when we pour slowly water into the jar and the sand becomes wet. From a certain point of view such reactions may be called memory, since the crab does the same as it has done under its natural conditions. We imitate those conditions as closely as possible and sometimes we get the same reaction. It is also not true that the fiddler closes its burrow "before the tide." It does it always *during the tide.* when the slowly rising water has moistened the air-chamber, not yet covering the surface of the ground. The arising slight movement of sand grains or the walls of the chamber becoming softer may be perceived by the crab as the beginning of the tide. As far as I observed *Uca* never remains a long time on the surface of the ground without visiting from time to time its burrow.

And thus, exactly as in heredity, not a given reaction in itself characterizes an animal, but always the faculty of producing this reaction *under given conditions.*

5. What becomes of the burrow after the tide? The tide destroys a part of the burrow and the crab must dig itself out. This may be done in several manners and I must confess that

the interpretation is very difficult. As mentioned above a quick pouring of water in the jar causes the walls to collapse, thus destroying the burrow completely. The animal forces itself through the sand and after the water has been removed it digs a new burrow in quite a new spot. But if the burrow was tightly closed *Uca* begins to work methodically, provided that the "tide" has gone out. Its movements are now somewhat the reverse of the ordinary burrowing. It will be remembered that the animal while remaining motionless in the air-chamber has its large chela directed to the inside. Now the outer legs, 2, 3, 4, grasp a sand pellet from the roof of the chamber and push it down towards the sagittal axis of the body. Then the crab climbs a little upward until the pellet lies between the carapace and the legs II., III., IV., which grasp it in turn. *Uca* comes down again and the sand will be deposited and kneaded at the bottom of the chamber. Still pursuing this work, the animal causes the chamber to rise slowly in an oblique direction, still keeping its volume unaltered, as the sand is always only carried from the roof of it to the bottom. At last the fiddler emerges, leaving the former canal filled with sand behind itself. Only the upper part of the burrow corresponding to the chamber in the last moment of work remains open. Now the crab may roam for a long time around the jar or it will start digging a new burrow at once. In most cases it works now along the same way, entering the hole from which it has emerged and following the same track. Usually it carries then the removed sand out of the burrow and the whole canal remains open. But, curiously enough, often will the crab close the canal behind it. After some time *Uca* is sitting again in the air-chamber at the bottom of the jar, while the whole burrow is tightly filled with sand, as if the animal wanted only to breathe fresh air for a while. In following the previous track the crab is evidently guided by the smaller resistance of the sand filling the burrow as compared with the surrounding. If the jar was filled with water for some time the sand collapses and there is no difference in its consistency. In this case *Uca* digs itself out once more, but does not follow exactly the previous track and sometimes it will dig in a perfectly vertical direction. In the apparatus

described above, where the fiddler had to dig between two glass plates, this exact following of the former track may be easily observed. A female dug a vertical hole (Fig. 3, *a*) and then the whole apparatus was turned at right angles. The crab changed the direction of the digging (Fig. 3, *b*). All the time the burrow remained filled with sand save the end-chamber. On the following morning I found the female out of the apparatus and the hole showed conditions as on Fig. 3, *c*. The track of digging could be distinctly followed and it corresponds exactly to the previous one.

Nevertheless such a behavior is not a rule and I have seen many times that the crab may choose an entirely new direction. One individual has dug itself out and the burrow remained open for several hours. Then it entered into it, grasped sand from the bottom and carried it to the opening, closing it in the usual way. *Uca* then proceeded to work until the whole canal became tightly filled with sand. During the work the end-chamber increased both in volume and length, directing itself obliquely upward but at right angles to the former burrow. After having closed the burrow the crab immediately started again the work of digging itself out in the new direction. It detached pellets from the roof and deposited them at the inner corner of the chamber. After some time a new open burrow was manufactured and as the end-chamber has increased considerably during the previous work it had now room enough for all sand detached from its roof. As a result there was a new open burrow at right angles to the first. I am unable to say what may be the biological significance of such a peculiar behavior.

We see clearly at least that closing the burrow before high tide and opening it at low tide by no means covers the whole field of the behavior of a fiddler crab. It is merely a scheme under which lies a whole world of varied, complicated and as yet perfectly incomprehensible activities.

6. The most difficult problem, of course, is the problem of interpretation. I do not know how to characterize the behavior of *Uca*. Is it reflex, instinct, or intellect? It will be probably safer to speak about the "activities" and it is certainly more important to investigate which are the real properties of those

activities than to reason about what they are and how to call them.

There is one striking property of very many reactions which I would like to insist upon: their plasticity. In fact, the closer we watch a fiddler the more obvious becomes the conclusion that there is very little automatic in it. The practical mode of accomplishing the various tasks depends on an infinity of minute circumstances which are so various and different that surely never two crabs work at their burrows under exactly the same conditions. The animal performs its tasks *in spite of* the condition being so various, and yet there is no doubt that every single movement is largely depending on external stimuli. When the pellet is carried out, the sand must be prevented from falling back into the hole. And we see that in every single case the sand is pushed, pulled and kneaded *until* it becomes properly adjusted. Yet the properties of a pellet, as its size, its form, the degree of moisture, the contents of organic matter and of clay, the position towards the walls of the burrow, towards the mouth of it, towards the other pellets, etc., are never the same. All those properties bear very strongly on the actual movements of the animal, which has to adjust not simply "a pellet of sand" but always *this single pellet* with all its individual particularities. The crab is always so careful about it that only rarely a few grains roll back into the hole and it must estimate and judge whether the work is done sufficiently well. Every single activity connected with burrowing may be analyzed in the same way. Choosing the spot where the burrow is to be made, grasping the sand, carrying the pellet out, turning in the burrow so as to make it circular, taking care of the burrow being conveniently bent, conveniently oblique, of a definite length, avoiding the obstacles, manufacturing the chamber, closing the burrow in various manners, adapting the mode of acting to a situation which is different in every successive second, each of those activities again may be performed in infinitely different ways. But they all bear on burrowing, which is only a small part of the whole behavior of a fiddler crab.

Given this unlimited variety of reactions, it becomes utterly impossible to admit that each single reaction is referable to a

particular fixed and unchangeable nervous mechanism. As may be deduced from the facts contained in the classical work of Bethe on the nervous system of *Carcinus*, this system is far too simple to be able to contain such a variety of reflex arcs. It does not follow from this that the activities may be controlled by something besides the nervous system. But it certainly follows from this that the morphology is of little help to us. We are compelled to admit a plasticity of the nervous centers; they must possess a certain creative power which enables them to become adapted to entirely new situations. The number of possible nervous connections is limited, but the number of possible reactions is infinite. This discrepancy may be avoided only by admitting that each nervous center may perform an infinity of functions.

SUMMARY.

The typical mode of digging a burrow is described and some problems discussed which are closely connected with the burrowing instinct.

The choice of the spot where the digging is started is determined by very many factors. Phototaxis and thigmotaxis are not sufficient explanations.

The end-chamber of the burrow functions as an air-chamber during the high tide.

The fiddler crab is a true water-breathing animal, but it can live in the air for several weeks without changing the water in the gill-chambers.

The length of the burrow partly depends on the degree of moisture of the ground.

There is no intrinsic periodicity in the life of *Uca*.

Several modes of closing the burrow and opening it are described.

In the interpretation the plasticity of activities is strongly insisted upon.

WOODS HOLE,
August, 1925.

LITERATURE.

1. **Abbott, J. F.**
'12–'13 The Effect of Distilled Water upon the Fiddler Crab. BIOL. BULL., Vol. 24.

2. **Alcock, A.**
'92 On the Habits of *Gelasimus annulipes* Edw. Ann. Mag. Nat. Hist., Vol. VI.
3. **Bethe, A.**
'97–'98 Das Nervensystem von *Carcinus Mænas*. Arch. f. mikr. Anat., Bd. 50, 1897, pp. 460 and 589. Ibid., Bd. 51, 1898, p. 382.
4. **Clark, G. P.**
'95–'96 On the Relation of the Otocysts to Equilibrium Phenomena in *Gelasimus pugilator* and *Platyonichus ocellatus*. The Journ. of Physiol., Vol. XIX.
5. **Cowles, R. P.**
'08 Habits, Reactions, and Associations in *Ocypoda arenaria*. Papers from the Tortugas Lab. Carnegie Inst. Wash., II.
6. **Dembowski, J.**
'23 Über die Bewegungen von *Paramœcium caudatum*. Arch. f. Protistenkde, Bd. 47.
7. **Dembowski, J.**
'23 Experimental biologische Studien über die Larve der Köcherfliege *Molanna*. Travaux de l'Inst. M. Nencki Varsovie., Vol. II. (Polish).
8. **Dembowski, J.**
'25 Experimentelle Untersuchungen über das Verhalten der Krabbe *Dromia vulgaris*. Teil I.–III. Ibid., Vol. III. (Polish).
9. **Frisch, K. v.**
'23 Über die " Sprache " der Bienen. Zoolog. Jahrb. Allg. Zool., Bd. 40.
10. **Garner, R. L.**
'92 The Speech of Monkeys. New York.
11. **Holmes, S. J.**
'08 Phototaxis in the Fiddler Crab and Its Relation to the Theories of Orientation. Journ. Comp. Neur. Psych., Vol. 18.
12. **Hyman, O. W.**
'20 Adventures in the Life of a Fiddler Crab. Ann. Rep. Smithson. Inst.
13. **Kingsley, S. y.**
'88 Something About Crabs. Amer. Nat., Vol. 22.
14. **Pearse, A. S.**
'13 Habits of Fiddler Crabs. Ann. Rep. Smiths. Inst.
15. **Pearse, A. S.**
'14 On the Habits of *Uca pugnax* (Smith) and *Uca pugilator* (Bosc). Trans. of the Wisconsin Acad. Sc. Arts. Lett., Vol. XVII.
16. **Rathburn, M. J.**
'18 The Grapsoid Crabs of America. U. S. Nat. Mus. Bull. 97.
17. **Schwartz, B., and Safir, S. R.**
'15 Habit Formation in the Fiddler Crab. Journ. of Anim. Behavior, Vol. 5.
18. **Schwartz, B., and Safir, S. R.**
'15 The Natural History and Behavior of the Fiddler Crab. Cold Spring Harbor Monographs, VIII.
19. **Symons, C. T.**
'21 Notes on Certain Shore Crabs. Spolia Zeylanica, Colombo, XI.
20. **Wasmann, E.**
'09 Die psychischen Fähigkeiten der Ameisen. 2 Aufl. Stuttgart.

THE PARTHENOGENETIC EFFECT OF SPERM FILTRATES, CONCENTRATED SPERM SUSPENSIONS, AND SERUM OF CHITONS ON THE OVA OF THE SEA-URCHIN, *STRONGYLOCENTROTUS FRANCISCANUS.*[1]

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Kupelwieser (1906) reported that the unfertilized eggs of two species of sea-urchins, *Strongylocentrotus purpuratus* and *Strongylocentrotus franciscanus*, exposed to concentrated sperm or to filtrates prepared from heated sperm of two species (?) of chiton, formed fertilization membranes; and, if subsequently treated with Loeb's hypertonic sea-water, developed. Loeb (1907 and 1908) obtained similar results with the blood serum of different species of animals. In recent experiments I have obtained similar effects with the ova of *S. franciscanus*. In addition filtrates prepared from suspensions of living sperm of the chiton, *Katharina tunicata*, caused the formation of fertilization membranes in ova of *S. franciscanus*. Subsequent treatment with hypertonic sea-water led to the development of many of the ova in which membranes appeared.

These experiments were performed during the spring of 1921 at the Hopkins Marine Laboratory at Pacific Grove, California. I wish to express here my appreciation of the hospitality extended to me at this laboratory and my thanks to the Director, Dr. Walter K. Fisher, for his generous assistance.

MATERIAL.

Within a few miles of the Laboratory, in protected situations along the rocky coast, there is to be found an abundance of the sea-urchins, *S. franciscanus* and *S. purpuratus* and of the chitons, *Katharina tunicata*, *Ishnochiton magdalensis* and *Cryptochiton*,

[1] Contributions from the Zoölogical Laboratory of Smith College No. 137.

which were used in these experiments. All of these occur in similar situations within Monterey Bay. Where easy of access they are not as abundant, because they serve as food for the Japanese and the Italians of the region. *S. franciscanus* is to be found from a few to many feet below low water line and *S. purpuratus* just below this line. On calm days both species can be seen at depths of twenty feet and hundreds of feet from shore. *S. franciscanus* is rarely exposed at the lowest tides, whereas *S. purpuratus* is commonly found in isolated tide pools or completely exposed at low tide. In many locations this species is so abundant that the rocks are carpeted with this purple sea-urchin. The chitons adhere to the rocks by their pedal muscles (Sampson, 1894), and are exposed only at the lowest tides.

Ripe gametes of *S. purpuratus* were available from December until June and continued to be so, according to Dr. Fisher, until the middle of July in individuals from certain locations. In sheltered parts of Monterey Bay all individuals shed during the week of March 26, following a series of heavy rains. During the first week of May a similar shedding occurred at "Parke Davis Point," a sheltered location on the Pacific coast. In the most exposed collecting area, shedding had not occurred before the middle of July. Elder (1911) and Loeb (1915) have recorded similar breeding habits for this species. Few individuals of *S. franciscanus* contained fully developed gonads before April. The unusually cold season probably accounts for their slow development, as Dr. Frank Lillie had obtained plenty of ripe individuals in January and February of the preceding year in similar locations. In the chitons ripe spermatozoa were present in March, but ripe eggs were not obtained until the first week in May. In *K. tunicata*, according to a personal communication from Dr. Gertrude Van Wagenen, eggs were not shed until late in July in exposed locations. Heath (1899) states that the eggs of *Ishnochiton* are shed in May and June.

Methods.

In the sea-urchins the oral disc was cut away; the contents of the body cavity, with the exception of the gonads, removed; and the body cavity thoroughly irrigated with filtered sea-water.[1]

[1] All sea-water was obtained directly from Monterey Bay and filtered. Because

The gonads were transferred to Syracuse watch glasses, in which they shed their reproductive cells.[1] The gonads of the chitons lie just beneath the dorsal shells, and were exposed either by removing the latter or by removing the muscles and the viscera ventral to the gonads. The body cavity was thoroughly washed with filtered sea-water and the gonads placed in Syracuse watch glasses. The body fluid was obtained by piercing the pedal muscles of the chitons and allowing the body fluids to drain out into finger bowls. These fluids were then filtered through double layers of filter paper.

The filtrate of sperm suspension of *K. tunicata* was prepared by filtering ten per cent. sperm suspensions of this species through tested Mandler filters according to the method used in another investigation (Sampson, 1926).

Results.

Exposure of the ova of *S. franciscanus* to a filtrate of a ten per cent. sperm suspension of *Katharina tunicata* for one to twenty minutes led to the formation of perfect fertilization membranes in the majority of the ova. If transferred to sea-water cytolysis occurred very rapidly in those eggs possessing membranes. If exposed for sixty to eighty minutes to hypertonic sea-water many of those with membranes developed into gastrulæ and plutei which were normal in appearance.[2]

In highly concentrated sperm suspensions of *K. tunicata* the ova of *S. franciscanus* formed membranes within one to three minutes. From forty to sixty per cent. of the ova were so affected. Within ten minutes these membranes were perfect in contour. Dilute suspensions of spermatozoa were ineffective. The spermatozoa of this species are inactive in sea-water and in

of copper in the pipes the running sea-water in the laboratory could not be used, nor could water be carried in galvanized iron pails. Enough zinc dissolved in the sea-water in transit to prevent the fertilization of eggs in it, or to prevent the fertilization of ova from females washed in such water.

[1] This proved to be the most satisfactory method of obtaining the reproductive cells from *S. franciscanus*, partly because of the large size of this sea-urchin and partly because the reproductive cells were so concentrated and so viscous that they did not shed readily through the genital pores.

[2] Ova of *Nereis* exposed to the sperm filtrate only of *Arbacia* formed membranes, maturated, and a small percentage of those with membranes segmented normally and developed into abnormal trochophores (Sampson, 1926).

egg-water of *S. franciscanus*, but are intensely activated in the actual presence of ova of the latter and form dense halos around them. Such eggs, freed from the excess of sperm by repeated washing in sea-water, fail to develop and those with membranes cytolyze in sea-water within twenty hours. If, however, the washed ova are subjected to a treatment with Loeb's hypertonic sea-water a certain percentage of those with membranes develop into gastrulæ and a smaller percentage into plutei. The best results were obtained when the ova were exposed to a concentrated sperm suspension for one and a half minutes and then to hypertonic sea-water for seventy minutes. At the end of sixty hours there were twenty-eight per cent. gastrulæ and seventeen per cent. plutei. The remainder had cytolyzed. The plutei lived for eleven days and the majority were normal in appearance. Similar results were obtained with concentrated sperm suspensions of *Ishnochiton magdalensis* and of *Cryptochiton*. Ova treated with hypertonic sea-water only for similar periods did not develop and such ova could subsequently be fertilized in sea-water with species-true sperm.

The blood serum of *K. tunicata* produced results similar to those obtained with sperm filtrates or with the concentrated sperm suspensions of this species. If concentrated serum is used the resulting membranes are wrinkled and unevenly elevated. If the serum is diluted twenty to thirty times normal membranes are produced. (Robertson (1912) obtained his best results with diluted serum in experiments with ox blood on ova of *S. purpuratus*.) The subsequent cytolysis in sea-water can be prevented and the development of many of those with membranes obtained if the ova are treated with hypertonic sea-water for seventy minutes after exposure to the serum. Similar results were obtained with dilutions of the serum of *Ishnochiton magdalensis* and of *Cryptochiton*.

The ova of *S. purpuratus* were used in similar series of experiments. In place of a typical membrane, ova exposed to test preparations, appeared to be surrounded by a swollen transparent jelly which tended to dissolve in sea-water. Such eggs could be fertilized in sea-water with sperm of the same species at once or two hours later. Such eggs if treated with hypertonic sea-water did not form membranes or develop.

Conclusions.

Ova of *S. franciscanus* exposed to sperm filtrates, concentrated sperm suspensions, or to blood serum of *Katharina tunicata* for one to three minutes formed typical fertilization membranes. Subsequent treatment with Loeb's hypertonic sea-water for seventy minutes caused a large percentage of those with membranes to develop into gastrulæ and plutei. Every precaution was taken to avoid contamination of the sperm suspensions of the chiton with body fluids or serum. It seems probable that the membrane formation in the filtrates and in the concentrated sperm suspensions is formed by some substance derived from the sperm.[1] The nature of this substance has not been determined. Experiments with filtrates of sperm of *Arbacia*, *S. purpuratus* and *S. franciscanus* and *Nereis* indicate that it is an organic compound (Sampson, 1926). The effect produced by the serum is probably due to organic compounds present in the latter.

Similar results were obtained with ova of *S. franciscanus* which were treated with concentrated sperm suspensions and with dilute serum of two other chitons, *Ishnochiton magdalensis* and *Cryptochiton*. With ova of *S. purpuratus* no typical membranes formed in the preparations used, and the fertilization capacity of the treated eggs appeared to be normal.

Literature.

Elder, J. C.
'11 Archiv. f. Entw.-mech., 35, 145–163.
Heath, Harold.
'99 Zool. Jahrbuch., 12, 567–657.
Kupelwieser, Hans.
'06 Biol. Centralbl., 26, 744–748.
'09 Archiv. f. Entw.-mech., 27, 434–462.
Loeb, J.
'07*a* Pfluger's Archiv., 118, 36–41.
'07*b* Univ. of Calif. Pub. Physiol., 3, 57–58.
'08*a* Pfluger's Archiv., 122, 196.
'08*b* Pfluger's Archiv., 124, 37.
'15 Amer. Nat., 49, 257–285.
Sampson, Lillian.
'95 Jour. Morph., 11, 596–628.
Sampson, Myra M.
'26 Biol. Bull., 50, April, 1926.
Robertson, T. B.
'12 Archiv. f. Entw.-mech., 35, 64–130.

[1] A membrane swelling has been observed in concentrated suspensions of sperm by Heilbrunn (Biol. Bull., 29, p. 149–203), and attributed by him to a substance produced by sperm.

VASODILATATION IN *FUNDULUS* DUE TO A COLOR STIMULUS.

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In a previous issue of the BIOLOGICAL BULLETIN,[1] the writer gave an account of the results of experiments with differently colored environments on *Fundulus*. These experiments were carried out in the Harvard Zoölogical Laboratory during the winter of 1922–1923. It was stated in that article, that the distinctive coloration obtained when *Fundulus* was placed in a red environment was due to a vasodilatation. It was further stated in a postscript that similar experiments were carried out elsewhere by the writer during the summer of 1924 with the same adaptive color changes occurring, but that no vasodilatation was observed when *Fundulus* was placed in a red background. It was suggested that the different results might be due to seasonal or local differences in the fishes.

During the past summer further tests were made at the U. S. Bureau of Fisheries Laboratory, Woods Hole, to determine the factors causing this difference. Positive results were obtained which will be briefly reported here.

In these experiments the fishes were first made to assume the light shade by being placed on a white background and from this stock they were selected for the various tests. Five specimens were placed in each of four battery jars containing fresh water and the jars placed in the differently colored boxes. Adaptation to yellow, blue, green, and red backgrounds were obtained with a vasodilatation occurring in specimens from the red background.

The experiments were repeated under the same conditions except that salt water was used. Adaptation to the various colored backgrounds occurred, but vasodilatation was not observed in the specimens placed in the red environment at the same period of exposure to the stimulus.

[1] Adaptive Changes in Shades and Color of *Fundulus*, BIOL. BULL., Vol. XLVIII., January, 1925.

To make a more precise comparison of the effects of a red environment, two tests were made at the same time under identical conditions except that in one set fresh water was used, in the other, salt water. When both sets were compared on a white surface on the third day, there was a decided difference in the appearance of the fishes, those in the salt water lacking the striking pink coloration. There was, of course, a general similarity in coloration, due to definite states of contraction of the chromatophores whether in salt or fresh water, but the pink condition due to vasodilatation was absent in the former case. There was however a slight vasodilatation even in the specimens placed in salt water, especially under a prolonged stimulus, yet not sufficient to give the distinctive pink coloration of specimens placed in fresh water. The salt water must therefore inhibit, though not completely, the vasodilatation.

The rapidity with which this vasodilatation occurs varies with the age and vigor of the fishes. In young vigorous specimens from 6 to 9 cm. in length, placed in fresh water it never fails to occur, generally on the second or third day of exposure to the red background, though it is frequently intensified by the fifth day. An adaptive change due to the movements of the chromatophores occurs much earlier being already noticeable in a few hours.

If the specimens from the red environment are immediately placed on a white surface in good light, the condition of vasodilatation can readily be observed. It is most noticeable about the head, especially in the two large vessels of the dorsal region; also at the end of the spinal column and the base of the fins. The peripheral vessels along the dorsal median axis of the trunk are also strikingly dilated. It is interesting to observe here, the rapid disappearance of the dilation. If one fixes the attention on definite peripheral vessels, the change will be noticeable within one minute, and in about two minutes the phenomenon of vasodilatation largely disappears.

A close approximation to the condition produced by the red environment in *Fundulus* was obtained with the use of adrenalin. The fishes were first made to assume the very dark shade by being placed on a black background until their melanophores

were maximally expanded. An intra-peritoneal injection of 0.50 to 0.75 cc. of adrenalin chloride—dilution 1 to 1000—was then given. In about 30 seconds a contraction of the melanophores was noticeable and this contraction, as judged by the unaided eye, was completed in about two minutes, when the fish assumed a pale grayish hue. In two to three minutes it began to assume a yellowish color, due to the partially contracted xanthophores in which a dense orange concentration of pigment might be seen on microscopic examination. In three to four minutes, a dilatation of blood vessels was noticeable at the base of the fins, especially the dorsal fin and about the head. The dilatation extended over the body generally within five or six minutes. In about twelve to fifteen minutes the coloration was similar to that obtained under the influence of a red environment, except that the adrenalin caused a more pronounced dilatation in specific regions as for example in the fins. The yellow color gradually diminished, but the vasodilatation persisted for hours. In one specimen the maximum degree of dilatation was observed five hours after the injection of adrenalin. The time periods for the different phases of the reaction varied slightly according to size and vigor of the fishes and the amount of adrenalin chloride injected. But those given above are typical of a dozen experiments on specimens 7 to 8 cm. in length.

If the caudal fin be examined under the microscope when the injection is made, it will be seen that there is an immediate constriction of the blood vessels causing a stoppage of circulation. This is soon followed by a renewal of the circulation which gradually extends over the fin. Adrenalin causes an immediate constriction and a post-dilatation of the blood vessels in *Fundulus* as in human beings. Apart from giving a convenient control for experimentation this post-dilatation effect of adrenalin is itself an interesting phenomenon in the light of a similar effect produced by a red environment.

SECRETION IN THE AMITOTIC CELLS OF THE CRICKET EGG FOLLICLE.

MARGARET R. MURRAY.

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I. INTRODUCTION.

The investigation of the cricket follicle-cell was undertaken primarily with the aim of observing its method of division (described by E. G. Conklin in 1903 as a type of the amitosis of senescence). Interpretation of the nuclear phenomena was, however, so greatly modified by study of the cytoplasmic phenomena paralleling them, that the nuclear division came to be considered rather as incidental to the other activities of the cell than as a manifestation important in itself. For the large amount of yolky substance with which the cytosome of the

ovarian egg is provided, is elaborated through the agency of the follicle cells. These assume intense secretory activity during the later stages of the oöcyte's development, a time coincident with their own period of amitotic division. This paper contains an account of the nuclear and cytoplasmic phenomena of the amitotic cells of the cricket egg-follicle, observed in specimens living and fixed, during their period of secretory activity.

The investigation was carried out for the most part at Washington University, Saint Louis, in 1923 and 1924 under the direction of Professor Caswell Grave. Additions and revisions were made at the Hull Zoölogical Laboratory of the University of Chicago in the fall of 1924, by the courtesy of Professor F. R. Lillie. It is a pleasure to take this opportunity of expressing my thanks to Dr. Grave for valuable suggestions and criticisms; and to Dr. Margaret Reed Lewis, for suggestions relating to the technique of tissue cultures.

II. MATERIAL.

The cricket ovary consists of a bundle of egg-tubes situated in the posterior part of the abdomen, ventro-lateral to the digestive tract, and communicating by means of a short oviduct with the vagina. Each egg-tube contains a number of eggs (usually from 8 to 12 at a time), arranged in linear series according to size; the largest and most mature egg being adjacent to the oviduct, and the smallest located at the opposite end of the egg-tube, connected distally with a "nest" of undifferentiated cells. (See Fig. 15.) The egg-tube, or follicle (which descends with the egg), is one layer of cells in thickness. Between the egg and its follicle a rather thick vitelline membrane intervenes which exhibits hexagonal markings that correspond with the outlines of the bases of the superimposed follicle cells. The follicle cells themselves are of an epithelial nature, and approximate hexagonal prisms in shape, varying in proportions with age (to be discussed more fully further on) from the columnar to the squamous type. Except at the proximal end, where it is attached to the oviduct, the egg tube lies within the hæmocoel of the insect; but contact with the blood is prevented by a thin, closely investing membrane.

The species of cricket used for study were, *Gryllus abbreviatus*,

the rather large, short-winged field-cricket; and *Nemobius fasciatus*, the small, striped ground-cricket. In practically all of this work, the two crickets could be used interchangeably, since the only differences between them so far as the ovarian tissue is concerned, involve the size of the egg-string as a whole, and the number of cells constituting an egg-follicle.

Since, in the history of any one egg, the stages designated in Fig. 15 as *A*, *B*, *C*, etc., form a continuous series, there is of necessity considerable overlapping between the conditions described for any two contiguous stages. In general, however, rapid and copious secretory activity is observed in the *A* and *B* follicles and to some extent also in the *C* follicle during September and October. Mitotic figures have been observed in cells of follicles distal to the *C* egg; only amitotic nuclear changes appear in stages *A*, *B*, and *C*.

III. METHOD.

The follicle cells were studied in tissue cultures with and without the use of vital stains. They were also studied after treatment with six fixatives and twelve stains, with their various possible combinations.

The culture medium most successfully used is a Locke solution as modified by Lewis and Robertson (M. R. Lewis and W. B. Robertson, '16):

Sea water	30 cc.
Distilled water	50 cc.
$NaHCO_3$	0.02 gram
Dextrose	0.25 gram

To this was added 0.1 gram of peptone. The tissue was placed in a hanging drop with a cover slip as growth support. In general, the whole egg string distal to the *A* egg was used in one implantation; the *A* egg was usually implanted separately. In spite of precautions against septic conditions, the cultures had an unusual tendency to become infected, especially with coccus and bacillus forms. The greater number of the cases of infection were probably due to the difficulty, at the time of implantation, of keeping the ovary and the dissecting instruments from contact with the contents of the digestive tract.

Healthy follicle cells have a very characteristic refraction and lie regularly arranged in the epithelial layer, as hexagonal prisms with slightly rounded corners. Dying or dead follicle cells exhibit a marked decrease in refractive properties, a more opaque appearance, and a loss of their regular arrangement, due usually to plasmolysis. In general, the cells remain in healthy condition from 48 to 72 hours after implantation.

The vital stains used were methyl green, neutral red, toluidine blue, brilliant cresyl blue, nile blue sulphate, and janus green, dissolved in the culture medium in concentrations varying from 1 in 20,000 to 1 in 5,000. The stains were toxic in greater or less degree; janus green, toluidine blue and nile blue sulphate were especially so.

The fixatives used were as follows: Bouin's; Flemming's (strong formula, with acetic); Da Fano's cobalt nitrate modification of Cajal's method for Golgi apparatus (C. Da Fano, '20); Regaud's fixative for mitochondria; formol; Kopsch's osmic acid impregnation for Golgi apparatus; and Champy's fluid modified for showing Golgi apparatus. For stains and combinations of stains with fixatives, see Charts I., II., and III. The fixatives served as controls for one another. The egg string distal to the *A* egg was sectioned longitudinally. The *A* egg could not successfully be sectioned longitudinally (for use in cytological study) due to the brittleness of the fixed yolk. Transverse sections were made of the *A* and *B* eggs. Sections varied in thickness from 2 micra (in material fixed by Regaud's method) to 5 micra (after fixation in Bouin's solution).

IV. OBSERVATIONS.

A. Cell Division.

Follicles were observed in culture medium for nuclear division. Specimens of the "amitotic" follicle fixed in Bouin's solution show what appears superficially to be a mechanism which might function in nuclear division, *i.e.*, a series of strands radiating from the plasmosome, with particles of chromatin often resting upon them (Fig. 2). It was expected that a study of tissue cultures might throw light upon these structures. To this end, follicle cells whose nuclei were in an elongated or constricted

COLOR REACTIONS OF *A* AND *B* FOLLICLE CELLS OF THE CRICKET, WITH VARIOUS FIXATIVES AND STAINS.

Fixative and Stain.	Mitochondrial Granules.		Small Yolk Globules.	Refractive Proximal Granules in Cytoplasm.	Large Yolk Globules.	Intermediate Yolk Glob.	Distal Droplets.		Nucleolus.	Chromatin Particles.
	In Nucleus.	In Cytoplasm.					Sheath.	Core.		
Regaud:										
$K_2Cr_2O_7$, Acid Fuchsin	—	Red	Red	—	Red	—	Red	—	Red	Dark-red
Acid Fuchsin, Methyl Green	—	Red	Red	—	Green	—	Red	—	Pink	Dark green
Iron hæmotoxylin	—	Black	Black	—	Black	—	Black	—	Gray	Black
Sudan III (alcoholic solution)	—	Deep orange	Deep orange	—	Pale-orange	—	Deep-orange	Orange	Clear-greenish	Clear-greenish
Nile blue sulfate	—	Lavender	Lavender	—	Blue	—	Purple	—	Blue	Dark-blue
Erlich-Biondi	—	Red-orange	Red-orange	—	Red-orange	—	Red	—	Red-orange	Red-orange
Da Fano:										
AuCl, $NaHSO_3$	Black	Black (some annular)	Brown→ black (some annular)	—	—	—	Black	—	—	—
AuCl, $NaHSO_3$, Delafield's hæm.	Black	Black	Brown→ black	—	Lavender	—	Black	—	Lavender	Lavender
Nile blue sulfate	Black	Black	Brown→ black	—	Blue	—	Black	Pale-blue	Blue	Blue
Sudan III (alcoholic sol.)	Black	Black	Brown→ black	—	Pale-yellow	Pale-yellow	Black	Orange	Clear-greenish	Clear-greenish

COLOR REACTIONS WITH VARIOUS FIXATIVES AND STAINS.

Fixative and Stain.	Mitochondrial Granules.		Small Yolk Globules.	Refractive Proximal Granules in Cytoplasm.	Large Yolk Globules.	Intermediate Yolk Glob.	Distal Droplets.		Nucleolus.	Chromatin Particles.
	In Nucleus.	In Cytoplasm.					Sheath.	Core.		
Da Fano: Erlich-Biondi	Black	Black (some annular)	Brown→black (some annular)		Red-orange		Black			
Osmic impregnation: Unstained	Black	Black	Black		Brown	Brown→ black	Black (present?)	Black	Greenish-yellow (black outline)	Greenish-yellow (outline of black granules)
Delafield's Hæmotoxylin	Black	Black	Black		Brown	Brown→ black	Black (present?)	Black	Brown	Brown
Delafield's Hæm., Methyl green	Black	Black	Black		Brownish-green	Black	Black (present?)	Black	Greenish-brown	Greenish-brown
Formol: Scharlach R (alc. sol.)	Orange		Orange			Orange	?	Orange		
Champy: Unstained	Black		Black	Golden yellow	Light-brown	Black	?	Black	Greenish	Greenish
Delafield's hæm., Methyl green	Black		Black	?	Dark-brown	Black	?	Black	Greenish	Greenish
Iron hæmotoxylin	Black		Black	?	Dark-brown	Black	?	Black	Brown	Black
Flemming: Iron hæmotoxylin					Black		?		Black	Black
Bouin: Delafield's acid hæm.			Rose	Pale rose	Reddish-brown		Reddish-brown		Red	Red
Iron hæmotoxylin, Eosin			Purplish-rose		Rose→black		Black		Black	Black
Erlich's hæmotoxylin					Purplish-rose		Dark-purple		Light-purple	Dark-purple

COLOR REACTIONS WITH VARIOUS FIXATIVES AND STAINS.

Fixative and Stain.	Mitochondrial Granules.		Small Yolk Granules.	Refractive Proximal Granules in Cytoplasm.	Large Yolk Globules.	Intermediate Yolk Glob.	Distal Droplets.		Nucleolus.	Chromatin Particles.
	In Nucleus.	In Cytoplasm.					Sheath.	Core.		
Bouin:										
Gentian violet, Gram's solution	—	—	Red	Pink	Pink→purple	—	Red	—	Red	Red
Gentian violet, safranin	—	—	Red	Pink	Rose	—	Red	—	Red	Red
Safranin, Licht-grün	—	—	Dark-red	Pale-pink	Rose→green	—	Dark-red	—	Bright-rose	Dark-red
Erlich-Biondi	—	—			Red-orange	—	Red	—	Red-orange	Red-orange
Sudan III. (alc. sol.)	—	—			Orange	—	Dark-orange	—	Greenish-yellow	Greenish-yellow
Nile blue sulfate	—	—			Blue	—	Bluish-green	—	Blue	Green
Tissue cultures:										
Janus green		Deep green					Pale-green	—	Green outline	
Methyl green									Green	Green
Brilliant cresyl blue		Blue?								
Neutral red							Red?	Red		
Toluidine blue							Blue	—		
Nile blue sulfate			Purplish-blue		Pale-red	Bright-red	Deep-lavender	Pale-red		

state at the time of implantation were drawn with camera lucida and observed from time to time for periods of which the maximum was five days; but no significant change in shape of either nucleus or nucleolus was noted in either stained or unstained cultures, although the procedure was repeated many times. It is possible that the nuclear strands may have changed slightly in relative position, but such a change was difficult to observe with accuracy. The cells of the *D* and younger follicles were found too small to be observed satisfactorily.

When it was ascertained that division could not be seen taking place in the cells of the *A* and *B* follicles, forty follicles of the large cricket were fixed and sectioned. Of this group, ten *A* follicles were sectioned longitudinally and ten transversely; and the number of cells in the largest section of each longitudinally-cut follicle was counted, as well as the number of cells in the largest section of each transversely-cut follicle. The same procedure was carried out for twenty *B* follicles. Results follow:

Fol. No.	*A* Egg. No. Cells in Largest Circum.		*B* Egg. No. Cells in Largest Circum.	
	Long. Sect.	Trans. Sect.	Long. Sect.	Trans. Sect.
1.....	192	43	220	68
2.....	186	62	166	69
3.....	180	67	232	64
4.....	180	62	204	68
5.....	190	68	184	60
6.....	200	76	200	60
7.....	184	72	238	59
8.....	202	65	174	62
9.....	196	61	220	58
10.....	190	48	140	70
Total.....	1,900	624	1,978	660
Average...	190	62.4	198	66

From the foregoing tables it appears that while there is considerable variation among individual follicles of each class as to their number of cells, the average number in the *B* follicle is similar to the average number of cells in the *A* follicle, although there is great difference in size between *A* and *B*. This indicates that during these two stages very little, if any, increase in the number of cells in the follicle occurs. Moreover, nearly all of

the nuclei in these stages, as well as in the *C* stage, are very much elongated and distinctly constricted, and contain two or sometimes more nucleoli (Figs. 1, 2, 4); but this elongation, when found in the *B* or *C* follicles, is always in a plane *perpendicular* to the surface of the follicle, so that the constriction if completed would divide the follicle into two layers, one beneath the other. In no follicle has such a state of delamination been observed; but when the follicle is approaching the *A* stage, many of the nuclei appear to have oriented themselves so that their axis of elongation is *parallel* to the follicular surface. Nuclei which are apparently in transition from one position to the other may be seen rather frequently (Fig. 8). Coincident with this shift in the position of the nucleus, a change in the shape of the follicle cell from a columnar to a squamous form takes place; so that from being elongated like the nucleus in a plane *perpendicular* to the follicular surface, the cell becomes expanded and flattened in a plane *parallel* to that surface, growing in absolute volume the while. This fact seems to account for an enormous increase in the surface area of the follicle as a whole, between the *B* and the *A* stages (occupying from one to two months), without cell division. The results of the tissue cultures indicate that the elongation and constriction of the nucleus and the division of the nucleolus is at most an extremely slow process. (Conklin, '03, suggests that frequent division takes place between the *D* and *B* stages, and that division is very slow in the *A* stage.)

B. Metaplasmic Inclusions of Follicle Cell and Egg.

1. *Distal Droplets.*

Unstained follicle cells in culture, used as controls, show compact and very highly refractive nucleoli, somewhat less refractive chromatin particles, and slightly refractive nuclear strands. The cytoplasm has a rather homogeneous and finely granular appearance, except for a number of large and exceedingly refractive granules which are usually present and, whenever found, appear in the part of the cell distal to the nucleus (Figs. 7, 8, 13). There is some, not a great deal, of variation from cell to cell in the numbers of these droplets, but in general they

are most numerous in the cells of the *B* stage of the egg tube. Within any given cell the distal droplets vary in size in a rather regular manner, the smallest being nearest to the nucleus,—often applied to its membrane, the largest being adjacent to the distal cell wall. When viewed from above, they often appear to form a ring just above the nucleus (Figs. 11, 12). This position, and the fact of their gradation in size seems to indicate a close relationship with the nucleus at the time of their origin.

In the living cell these droplets stain red all the way through with neutral red. But with toluidine blue and with janus green only the outer rim takes on color. Nile blue sulphate has been demonstrated by J. L. Smith to stain fats differentially; *i.e.*, neutral fats are stained red, and fatty acids blue, the degree of acidity being indicated roughly by shades of lavender. When the egg string is stained with nile blue sulphate, the distal droplets show a pale red medulla with a deep lavender cortex. The droplets stain as a whole with scharlach R after fixation in formol; they are blackened after impregnation with osmic acid, and after treatment with Champy's mixture of osmic and bichromate. When tissues fixed with osmic acid are later treated with turpentine, the medulla dissolves first, leaving an indistinct gray base; the cortex dissolves only after long exposure. After other treatments, such as Regaud's, Bouin's, and Da Fano's, the medullary portion is dissolved out in the alcohols, leaving only a thin base which will take certain stains (see charts); but the cortical portion is preserved, usually as a ring of very small droplets.

The distal droplets were observed for indications of movement, at various periods after the explantation of the egg string in unstained media and in media stained with nile blue, toluidine blue and methyl green respectively. Movement of the distal granules was found to occur at all periods, and to occur so rapidly during the first four or six hours after implantation, as to be almost perceptible. These droplets, which were congregated in the distal region of the follicle cell, were observed with the low power to pass through the external membrane of the egg string. Shortly after implantation, the passage occupied on the average about three minutes; *i.e.*, three minutes were

consumed between the time when the first suggestion of a droplet appeared on the outer surface of the external follicular membrane, and the time when the exuding droplet became perfectly spherical and separated itself from the membrane and lay free in the culture medium. The fact that the droplets pass out so readily indicates that the membrane is coarsely porous. It should be added that the cultures observed for the movement of the distal granules were uninfected and in good condition otherwise, hence the activity recorded is presumably a typical one, and not a result of fatty disintegration of the follicle cells. Moreover, the number of distal droplets in the cells does not tend to increase with the age of the culture, but rather to decrease. This decrease is coincident with the gradual decrease in the food and oxygen supply afforded by the medium.

By means of fixation and of vital staining, a histo-chemical identification of these cell inclusions is somewhat dubious. There may, however, be some value in suggestions as to what class of compounds the various inclusions belong. To this end the Table of Chemical Constitution of Fatty Substances (by Dr. W. Cramer in Lee's "Vademecum," page 358), Kingsbury ('11), Bell ('14), Bowen ('19), Smith and Mair ('10), and other works have been consulted with reference to the charts of the color reactions of these inclusions. The constitution of the medulla of the distal droplets seems to be something in the nature of a true fat, mixed, or perhaps combined, with a little protein, as suggested by the grayish base left after treatment with solvents. All the reactions of the medulla except its ready solution in turpentine after osmic acid fixation might be interpreted as the reactions of Golgi bodies. The latter, however, relegates it to some other group of lipoids, as does its passage into the hæmocoel. The cortex reacts as a lipoid with the lipoid fixatives, but is preserved through the alcohols after treatment with Bouin's fluid.

As to the function of the distal droplets in the life of the organism, I have as yet little or no indication. It is possible to conceive of them as an endocrine secretion from the follicle, although this seems unlikely in the insects; or, they might be merely excretory products.

2. *Mitochondria.*

In addition to the distal droplets, the cytoplasm of the follicle cell contains usually large numbers of small spherical granules, which are most numerous in the proximal region, but are found distally also. There is always a row of them applied to the nuclear membrane. These granules I shall speak of as mitochondria, since they conform in general to current descriptions of the form and distribution of cell inclusions to which that name is given, and since they stain red with acid fuchsin or black with hæmotoxylin after the Regaud treatment (Fig. 1), and stain with janus green in cultures (Figs. 9, 10). They are blackened with osmic acid (Fig. 5), and with silver nitrate (Fig. 4). Bouin's, Flemming's (acetic), and Champy's treatments do not preserve them (Figs. 2 and 3). Their numbers conform with the observations of Cowdry that abundant mitochondria are associated with intense protoplasmic activity. If such a relationship be granted, there are in these tissues indications of waves of protoplasmic activity: in many follicles, notably those impregnated with osmic acid, groups of cells which show a large number of mitochondria are contiguous with groups showing a lesser number, and these in turn with groups showing more, etc. Since the egg tubes are quite discrete, there can be no question of differential penetration of the fixative.

These mitochondrial granules are continuous in position, however, with granules in both the yolk and the nucleus of the follicle cell. In these three places they seem to be *generally* similar in lipoid nature as detected by staining reactions, but not *exactly* similar (see charts). In many cases, suggestively oriented rows of them can be seen, some members of which are in the vitelline membrane, and others, exactly similar in appearance, are beyond the membrane, within the egg (Fig. 1). Beginning a short distance within the vitelline membrane, they are found characteristically grouped around the other yolk constituents. In the Da Fano preparations, granules can be seen in the plasmosome of the follicle cell, and also in rows along the nuclear strands which radiate from it (Fig. 4). This is reminiscent of Saguchi's findings in the frog pancreas. It also suggests that the nuclear strands in the follicle cells may have a

metabolic significance. Essentially the same appearance is produced by impregnation with osmic acid (Figs. 5, 6): granules are not seen *within* the plasmosome, but form a sharp cortical sheath around it. In Champy preparations (Fig. 3), black granules are seen on the plasmosome, as well as surrounding it. (Here the plasmosome also contains clear yellow granules, somewhat larger than the black ones.) With scharlach R after formol, granules sheathing the nucleolus and scattered in the nucleus stain orange.

These observations seem to indicate a sequence or metamorphosis of granules from nucleolus of follicle cell to yolk of egg, with the mitochondrial state as one stage in the sequence. When they have reached the egg cytosome, the granules probably affect in some way the condition, or perhaps the synthesis of the other types of yolk globules, which they invariably surround. (Cf. Gatenby on Insect Ovogenesis.) It would, of course, be very desirable to see this process going on in the tissue cultures. This was attempted without success. It seems possible to attribute the failure partly to the opacity of the follicle cells (it was impossible to use either camera lucida or projection microscope on the small granules in order to detect motion over a long period of time); and partly to the extreme slowness with which the process must take place (August to November to produce the two to three cubic milimeters of yolk which are found in a mature egg).

3. *Yolk Globules.*

The arrangement of yolky materials in the oöcyte is a very definite one, as illustrated by the figures, especially Fig. 1, which represents an area extending from the external follicular membrane to the longitudinal axis of the egg. The yolk globules are oriented in the pattern indicated, with reference to the *periphery of the egg*, that is, with reference to the follicle cells. The nucleus of the egg is often distinctly eccentric, but that does not alter the arrangement of the yolk with reference to the follicle. Besides the small mitochondrial, or fuchsinophile, granules already referred to, the egg cytosome contains two types of globules. One of these, which will be referred to as the "large" type of globule, is at its maximum size a short

distance from the vitelline membrane in a moderately ripe egg (Fig. 1); in a thoroughly ripe egg the large globules are nearly all of the same size. The large type of globule appears red after fixation by Regaud's formula and stains with acid fuchsin. If methyl green is then applied, the red color is lost, and a green color taken on. Nile blue sulphate after silver nitrate impregnation stains these globules. They are colored a dark brown by osmic acid, stained a pale red by nile blue sulphate (in culture), and left unstained by scharlach R after formol. They are colored a very light brown by the Champy treatment. They are preserved by Bouin's and Flemming's fixing fluids containing acetic acid. It is suggested that these globules may be of a constitution on the order of lecithin.

There is some indication from tissues fixed in Bouin's and Champy's fluids, of the origin of the large type of yolk globule. Clear granules can be seen in the proximal part of the follicle cell, often forming rows from nucleus to cell membrane, and continuous with an increasing series of this type of yolk globule (Figs. 2, 3). The staining reactions inside, and outside, the cell membrane, are somewhat different. In the yolk mass these globules are preserved and stained with great readiness; the globules in the follicle cell take little color from the ordinary stains.

The other type of yolk globule, called "intermediate" in size, and represented in Figs. 1–4, is distributed in much the same pattern as the "large" type, but does not have as great a size-maximum. It is dissolved by either fixative or alcohols during all the treatments except Kopsch's and Champy's (in which it is blackened), and formol (after which scharlach R stains it a deep orange). Nile blue sulphate, used in the cultures, stains it a bright red. Turpentine dissolves this type of globule after osmic acid impregnation. It is probably, therefore, very close to a true fat in composition. The intermediate type of globule is always found surrounded by a fuchsinophile cortex—usually granular; but no evidence of a direct origin from the follicle cells has been observed for it, although it is evident that the materials of which the globule is synthesized must, like the others, reach the egg by way of the follicle cells. In the proximal

region of some of these cells there has been seen a nebulous structure composed of very finely divided black particles, which might be considered to indicate an excessive deposition of fat in the tissue. This fine emulsion might conceivably be coarsened at the boundary of the cell, and coalesce into fatty globules. (See Fig. 5.)

What part the egg itself may play in the synthesis of these yolky materials has not been ascertained. It is remarkable that no substances which could be identified as Golgi bodies have been found in either follicle cell or egg, although several standard fixatives for Golgi bodies have been used, and many kinds of inclusions preserved. With the use of either the Champy or the Kopsch preparation alone, doubtless the distal droplets would have been considered to be Golgi bodies. But all the observations combined make such a diagnosis doubtful for these or any other observed inclusions, in either egg or follicle.

V. DISCUSSION.

A. Significance of Nuclear Constriction.

A general discussion of the significance of amitosis and a review of the literature on the subject will not be attempted here in view of the full surveys afforded by Conklin (1917), Nakahara (1918), and Bast (1921).

Study of cell division in the "amitotic" follicle cells of the cricket has shown that in this form the division of the nucleolus and constriction of the nucleus rarely if ever are followed by the constriction and division of the cytoplasm; it has also shown that complete cell division is not necessary to account for the increase in the size of the follicle between the *B* and the *A* stages. That the amitotic behavior of the nucleus here is an indication or result of senescence seems open to question in view of the fact that at this time the follicle cells are metabolizing at a high rate in the elaboration of materials which go to make up the yolk of the egg, and therefore cannot, so far as metabolism is concerned, be called senescent. They are, it is true, highly differentiated cells, and in that sense the term senescent may be applied to them. But it seems to the writer that this amitotic behavior, confined as it is to the nucleus, should be given a

more particular interpretation, when it is found in connection with intense secretory activity, in which the nucleus plays so large a part as in these follicle cells. The proportionately great increase in nuclear and nucleolar surface which is the result of amitosis in these cells would appear to be a distinct advantage in their secretory activities, and might represent one phase in the cells' differentiation. Indeed the behavior of these cells bears little resemblance to the usual processes of cell division.

B. Relationship of Mitochondria with Yolk.

It is not generally conceded by American cytologists that mitochondria actually metamorphose into the constituents of yolk, or of other secretions; nor is it even generally held that these bodies play an intimate part in the production of secretions by secretory cells. Lewis and Lewis (1915), in a review of the literature on mitochondria and a description of their own observations of mitochondria in tissue cultures, state that they find no direct relation between mitochondria and the formation of fat. In a summary (1916) of the functional significance of mitochondria, Cowdry quotes Mathews (1915) in saying that mitochondria are related to the phospholipins found in all cells, whose function undoubtedly is to produce, with cholesterol, the peculiar semi-fluid, semi-solid state of protoplasm. Kingsbury (1912) describes mitochondria as intermediate products of metabolism, the structural expression of reducing bodies in respiration.

There seems, however, to be some evidence in the cricket egg that the mitochondria play here a rather specialized role in secretion. The fuchsinophile granules of the yolk, whether derived from the mitochondria of the follicle cells, as suggested, or not, are very similar to them in form and staining reactions; and are indistinguishable from any mitochondria which might be native to the egg. These are very intimately associated with the two larger kinds of globules in the oöcyte. Very similar granules also are found in the position of a cortex around the distal droplets. Although the resemblance, as regards staining, between the mitochondria of the follicle cell and the granules in these two locations is not exact, it is nevertheless so

close as to support the presumption that they have the common origin which their form and distribution suggests.

The work of Gatenby and his associates, on insect ovogenesis and other invertebrate problems, has led them to similar conclusions as to the rôle of mitochondria in secretion. In a 1920 paper, Gatenby and Woodger go so far as to state that in nearly all eggs where fat granules are present, examination has revealed that such "yolk" is derived either from Golgi elements or from mitochondria. It seems possible that some of the present divergence of opinion as to the function of mitochondria in the cell is due to the diversity of the material used in the investigation of the problem. The absence in the cricket follicle cells of inclusions which will satisfy the assembled criteria of Golgi bodies, is another case in point.

VI. SUMMARY.

1. Follicle cells of the *B* stage rarely if ever divide during the development of the oöcyte up to and through the *A* stage, although these cells change in size and shape during that period.

2. The amitotic configuration of the nucleus may be regarded as an adaptation to the secretory activity of the cell, having as its main function the increase of nuclear and nucleolar surface.

3. Droplets of a fatty nature are elaborated by the follicle cells and passed in large numbers into the hæmocoel of the insect.

4. The mitochondria of the follicle cells represent an intermediate stage between lipoid granules appearing in the nucleus of the follicle cell and lipoid granules surrounding the yolk globules of the egg.

5. No inclusions corresponding entirely to current descriptions of Golgi bodies have been observed in egg or follicle cell.

LITERATURE.

Bast, T. H.

'21 Types of Amitosis in Bone Cells. Amer. Jour. Anat., XXIX., September.

Bell, E. T.

'14 Differential Staining of Fats. Jour. Pathol. and Bact., XIX.

Bowen, R. H.

'19 New Methods for Analysis of Cytoplasmic Structures. Proc. Soc. Exp. Biol. and Med., XVII.

'22*b* Idiosome, Golgi and Acrosome. Anat. Rec., XXIV.

Coghill, G. E.

'15 Preliminary Studies on Intracellular Digestion and Assimilation in Amphibian Embryos. Science, XLII.

Conklin, E. G.

'03 Amitosis in the Egg-follicle Cells of the Cricket. Amer. Nat., XXXVII.

'17 Mitosis and Amitosis. BIOL. BULL., M. B. L., XXXIII.

Cowdry, E. V.

'14 Cytoplasmic Constituents of Nerve Cells. Amer. Jour. Anat., XV.

'16 General Functional Significance of Mitochondria. Amer. Jour. Anat., XIX.

'18 Contrib. Carnegie Inst., VIII.

'24 Value of the Study of Mitochondria in Cellular Pathology. Amer. Nat., LVIII.

Dafano, C.

'20 Golgi in Nervous and Other Tissues. Jour. Roy. Mic. Soc., pp. 157.

Gatenby and Woodger.

'20 On the Relationship between the Formation of Yolk, and the Mitochondria and Golgi Apparatus during Oögenesis. Jour. Roy. Mic. Soc.

Kingsbury, B. F.

'11 Histological Demonstration of Lipoids. Anat. Rec., V.

'12 Cytoplasmic Fixation. Anat. Rec., VI.

Kopsch.

'02 Die Darstellung des Binnennetzes in spinalganglionzellen wanderen Korpzellen mittelst Osmiumsaure. Sitzungsber d. k. preuss. Akad. d. Wiss., 40, 1.

Lee, A. Bolles.

'21 Microtomist's Vademecum. P. Blakiston's Son and Co., Philadelphia, Pa.

Lewis and Lewis.

'11 Cultivation of Tissue from Chick Embryos in Solutions of $NaCl_2$, KCl, and $NaHCO_3$. Anat. Rec., V.

'15 Mitochondria in Tissue-cultures. Amer. Jour. Anat., XVII.

Lewis, M. R., and Robertson, W. R. B.

'16 The Mitochondria and Other Structures Observed by the Tissue Culture Method in the Male Germ-cells of *Chorthippus curtipennis* Scudd. BIOL. BULL., M. B. L., XXX.

Lewis, W. H.

'22 Endothelium in Tissue-cultures. Amer. Jour. Anat., XXX.

Ludford, R. J.

'22 Behavior of Golgi During Amitosis. Q. J. Mic. Soc., LXVI.

Macklin, C.

'16 Amitosis in Cells Growing in Vitro. BIOL. BULL., M. B. L., XXX.

Matthews, A. P.

'15 Physiological Chemistry. William Hood and Co., N. Y.

Nakahara, W.

'18 Studies of Amitosis in Adipose Cells of Insects. Jour. Morph., XXX.

Nassanov, D. N.

'23 Das Golgische Binnennetz und seine Beziehungen su der Sekretion. Untersuchungen uber einige Amphibiendrusen. Arch. f. mikr. Anat. Berlin, XCVII.

Pappenheimer, A. W.

'16 The Golgi Apparatus. Observations and review. Anat. Rec., XI.

Regaud, Cl.

'10 Etude sur la structure des tubes séminifères et sur la Spermatogénése chez les mammifères. Arch. d'Anat. Micr., T. XI.

Saguchi, S.

'20 Studies on the Glandular Cells of the Frog's Pancreas. Amer. Jour. Anat., XXVI.

Smith, Christianna.

'20 Study of the Lipoid Content of Kidney Tubules. Amer. Jour. Anat., XXVII.

Smith, J. L., and Mair, W.

'10 Further Observations on the Bichromate Hæmotoxylin Method of Staining Lipoids. Jour. Path. and Bact., XV.

Wilson, E. B.

'00 The Cell.

Ziegler.

'91 Die biologische Bedeutung der amitotischen (direkten) Kernteilung im Tierreich. Biologisches Centralblatt 1891.

PLATE I.

Explanation of Figures.

1. Follicle-cells and a part of the *C*-egg, fixed by the Regaud method, and stained with acid-fuchsin and methyl green. Longitudinal section 3 micra in thickness; cells 1.67 micra in length. Drawn with camera lucida; enlarged to four times the area. Fuchsinophile granules in cytoplasm of follicle-cells and in cytosome of egg are figured black; globules staining with methyl green are stippled; dissolved globules are left white.

2. Follicle-cells and a part of the cytosome of the *B*-egg, fixed in Bouin's fluid and stained with Delafield's acid hæmotoxylin. Transverse section 5 micra in thickness; cells 3 micra in length. Drawn with camera lucida. Refractive granules of the proximal region of the cytoplasm are stippled more lightly than the large type of globules in the yolk substance.

3. Follicle-cells and a part of the yolk-substance of the *B*-egg, fixed in Champy's mixture of osmic and bichromate and pyrogallic acid; unstained. Longitudinal section 4 micra in thickness; cells 2.5 micra in length. Drawn with camera lucida. Two kinds of granules are shown in the nucleolus; refractive granules are shown in the proximal region of the cytoplasm of the follicle-cells.

Symbols.

d.g., distal droplet. *f.g.*, fuchsinophile granules. *i.g.*, intermediate globule. *l.g.*, large type of globule. *n.s.*, nuclear strand. *v.m.*, vitelline membrane.

Fig. 1

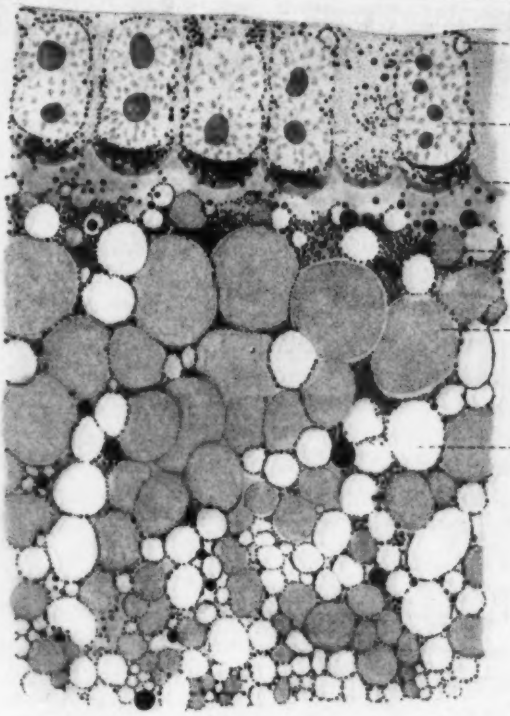


Fig. 2

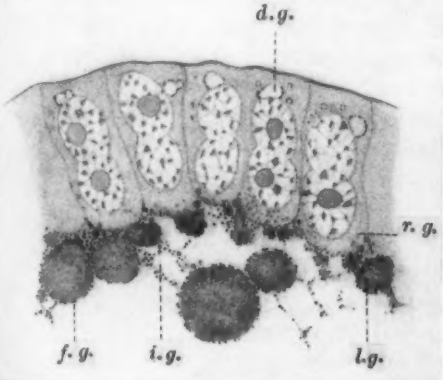


Fig. 3

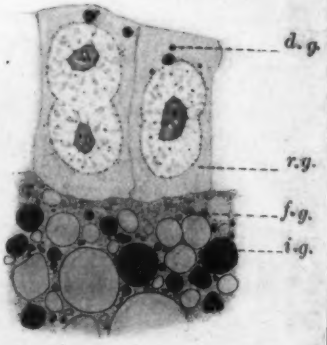


PLATE II.

Explanation of Figures.

4. Follicle-cells and a part of the cytosome of the *C*-egg, fixed by Da Fano's modification of Cajal's method for Golgi apparatus, and stained with Nile blue sulfate. Longitudinal section 4 micra in thickness; cells 1.5 micra in length. Drawn with camera lucida.

5. Follicle-cells of the *B*-egg, fixed by Kopsch's osmic acid impregnation method; unstained. Longitudinal section 3 micra in thickness; cells 3.5 micra in length. Drawn with camera lucida. Lighter granules in the cytoplasm are of the same character as the black ones, but lie at a lower level.

6. Follicle-cells of the *B*-egg, fixed by Champy's method, and stained with Delafield's hæmotoxylin. Longitudinal section 3 micra in thickness; cells 3.5 micra in length. Drawn with camera lucida. The nucleus tends to lie in the proximal region of the cell, while the distal region is occupied by a large number of granules.

Symbols.

d.g., distal droplet. *f.g.*, fuchsinophile granules. *i.g.*, intermediate globules. *l.g.*, large type of globule. *n.s.*, nuclear strand.

Fig. 4

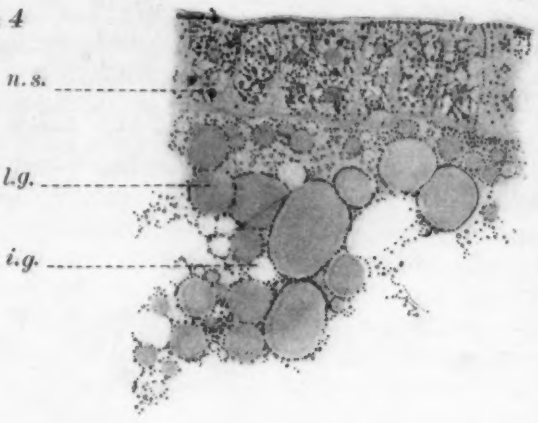


Fig. 5

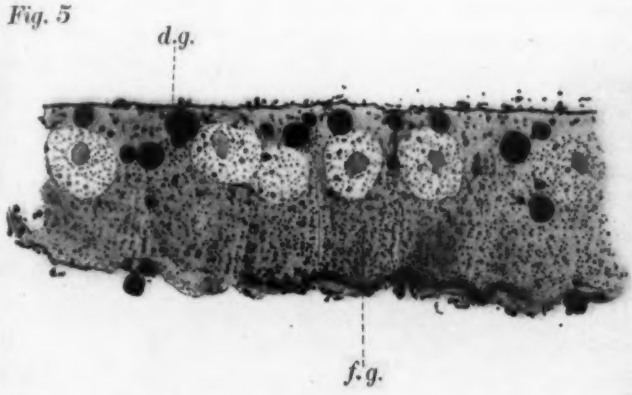


Fig. 6

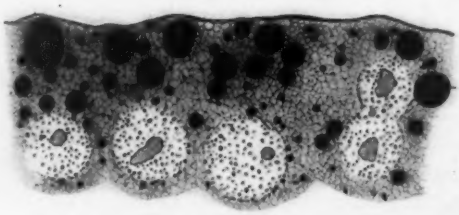


PLATE III.

(All drawings made approximately to scale.)

7. Diagrammatic sketch of a follicle-cell of the *B*-stage, unstained. The nucleus lies in the proximal region of the cell, and a large number of refractive granules occupy the distal region.

8. Camera lucida sketch of a cell from the *B*-follicle, unstained. The nucleus is in transition between the position in which its long axis is *perpendicular* to the surface of the follicle and the position in which it is *parallel* to the surface.

9. Diagrammatic sketch of a cell in the *C*-follicle, stained with janus green. Green-staining granules are shown outlining the nucleus and lying in the proximal region of the cytoplasm.

10. Surface view of a similar cell under the same conditions.

11. Camera lucida sketch of surface view of cells from the *A*-follicle, stained with neutral red and brilliant cresyl blue. The distal granules take the red stain.

12. Camera lucida sketch of surface view of cells from the *B*-follicle, stained with neutral red. The distal granules are red.

13. Camera lucida sketch of a cell from the *A*-follicle, unstained. The nucleus occupies the proximal region of the cell, and a number of highly refractive granules occupy the distal region.

14. Camera lucida sketch of surface view of a cell from the *A*-follicle, stained with methyl green. The distal granules are not stained. Some of them show small lobular irregularities, as if they were being formed by the coalescence of several smaller granules.

15. Camera lucida sketch of a whole egg-string. The black outline surrounding each egg represents the follicle. Beginning with the ripest in the series, the eggs are lettered in the order of their maturity. The *B*-follicle is the thickest of the series.

Fig. 7

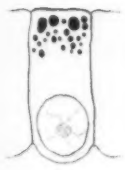


Fig. 8

Fig. 15

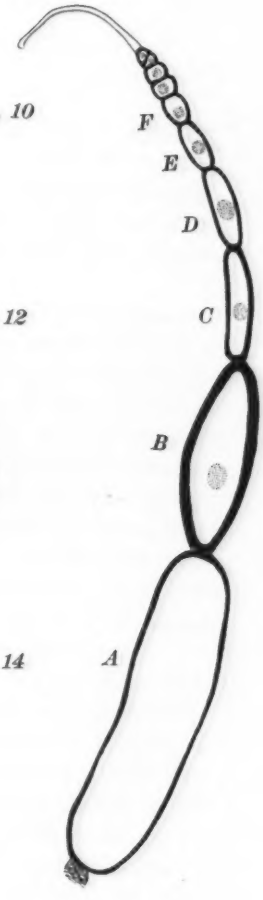


Fig. 10

Fig. 9

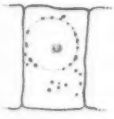


Fig. 12

Fig. 11

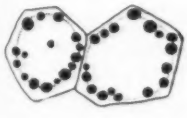
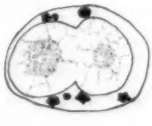


Fig. 13

Fig. 14



VITAL STAINING AND REDUCTION OF VITAL STAINS BY PROTOZOA.

ELERY R. BECKER,

IOWA STATE COLLEGE.

It is well known that fresh animal and plant tissues and milk have the power of reducing methylene blue to the leuco-base. This is supposed to be brought about by the hydrogen liberated in the decomposition of water by a reducase. The reducase in turn requires the presence of Schardinger's enzyme, an oxidizable substance, and water. (See Bayliss, p. 588.)

It is not generally known that protozoa, too, may possess the reducase. The demonstration of its presence requires that they be kept in a weak solution of the dye in an oxygen free medium. The method used by the writer was a hanging drop of ciliates and dye solution in an Engelmann chamber, through which a stream of hydrogen was passed. The drop was observed through the compound microscope. The ciliates used were *Opalina sp.?* from the bullfrog tadpole and *Paramecium caudatum*, representing both parasitic and free-living modes of life. Fifteen dyes were tested out on *Opalina*, and one on *Paramecium*. The best reductions are obtained if the hydrogen gas is first passed through heated platinized asbestos to remove the oxygen.

Janus Green.—Opalina in suspension of tadpole feces from posterior intestine of tadpole were suspended in a drop of Janus green (1 to 15,000) made up in 0.3 salt solution. Apparently the dye did not stain any substance in the ciliate. After displacing the air in the chamber with hydrogen, a number of the *Opalina* contain pink granules, due to reduction of the Janus green (diethylsafraninazodimethylanilin) to diethyl safranin. On exposure to air the pink substance did not change its color. Further reduction to the leuco-base could not be effected. Not over one fourth of the *Opalina* at any one time could reduce the dye. The reduction was also accomplished in a muscle chamber.

Brilliant Cresyl Blue.—Stains *Opalina* light blue in dilutions of 1 to 50,000. In the reduction to the leuco-base, there is an intermediate greenish hue. All the *Opalina* took the stain and showed reduction of the dye. On readmittance of air, the blue reappeared.

A number of *Nyctotherus* stained blue with this dye, but showed no reduction phenomena.

Nile Blue.—Stains *Opalina* blue-green in dilutions of 1 to 30,000. On reduction to the leuco-base the color disappears. Readmittance of air causes green stains to reappear.

Toluidine Blue.—Stains *Opalina* violet. Reduced to leuco-base. Color reappears on readmittance of air. Not all organisms took the stain.

Bismarck Brown.—Does not stain *Opalina.*

Methyl Green (*Vital*).—Living *Opalina* do not stain, even in dilutions of 1 to 1,000. In dilutions of 1 to 300 the dead ones stained a beautiful violet, but showed no reduction of the dye.

Vital Red.—1 to 2,000 does not stain *Opalina.*

Isamine Blue.—Does not stain *Opalina* in dilutions of 1 to 15,000 and 1 to 20,000.

Trypan Blue.—Does not stain *Opalina* in dilutions of 1 to 10,000 and 1 to 20,000.

Trypan Red.—Same results as *Trypan blue.*

Carminate of Sodium.—Same as *Trypan blue.*

Fuchsin (*Neutral*).—Same as *Trypan blue.*

Rhodamine.—In dilutions of 1 to 20,000 this dye will stain *Opalina* a light pink. No reduction of this dye could be accomplished, although in a test tube the dye could be reduced to the leuco-base by means of zinc and HCl.

Neutral Red.—Stains *Opalina* and certain granules and food vacuoles in *Paramecium* pink, but no reduction could be accomplished in the hydrogen chamber.

Methylene Blue.—This dye stains *Opalina* blue almost at once. It takes about ten minutes in order for it to stain *Paramecium* well. A comparison of the rate of reduction of the dye by the two ciliates would have been interesting, but the problem was complicated by the fact that *Opalina* completely and uniformly reduced the dye with which it is stained to the leuco-base within

three or four minutes, while *Paramecium* does not show so uniform a reduction. The dye is retained for a considerable length of time about the food vacuoles and granular substances in the protoplasm. This makes exact comparison of the rates of reduction difficult. But it is evident that the general protoplasm of *Paramecium* has become quite colorless at about the time the *Opalina* has reduced the methylene blue to the leuco-base. This fact makes it improbable that the *Opalina* has any advantage over *Paramecium* in its parasitic mode of life in the way of obtaining oxygen for respiration from the decomposition of water.

In addition to these experiments with *Opalina* and *Paramecium*, Janus green of about 1 to 15,000 dilution was tested out on the protozoa of the intestine of the termite *Reticulitermes flavipes*. These protozoa ingest the particles of wood which the termite eats. Cleveland (1924) has supplied the final proof that these protozoa are instrumental in assisting the termite to digest its cellulose diet. After a short time the Janus green stains the small particles of wood in the bodies of the various protozoa a light green. If the coverslip is sealed tightly with vaseline, the color changes to pink, and finally to the leuco-base. This suggests the possibility that oxidation of some of the products of digestion of the wood takes place in the vicinity of the food vacuoles, or that oxygen is used in some way in the digestion of the wood itself.

Conclusion.

Opalina has the power of reducing the vital dyes Janus green, brilliant cresyl blue, Nile blue, toluidene blue, and methylene blue. *Paramecium* shows similar reduction properties. This shows the presence of a reducase in the protoplasm of protozoa. The wood-ingesting protozoa of the termite's intestine possess a reducase in the food vacuoles, as proved by the reduction of Janus green in these parts of the cell.

The possession of this reducase is not an adaptation to the parasitic mode of life in the intestine where the oxygen pressure is low for free living protozoa may also possess it. The results are too meagre to state definitely whether the parasitic protozoa

may have the advantage over free living protozoa in possessing greater amounts of it.

My thanks are due to Professor E. N. Harvey, of Princeton University, for his suggestions in regard to the apparatus.

REFERENCES.

Bayliss, W. M.

'15 Principles of General Physiology. London.

Cleveland, L. R.

'24 The Physiological and Symbiotic Relationships between the Intestinal Protozoa of Termites and their Host, etc. BIOL. BULL., 46: 177–225.

THE INTERRELATIONS OF PROTOZOA AND THE UTRICLES OF UTRICULARIA.[1]

R. W. HEGNER.

References to the presence of "Infusoria" within the utricles of Utricularia occur in several publications on insectivorous plants, but no one has hitherto studied the interrelations of these protozoa and the plants. The situation suggests various questions, such as (1) are the protozoa intruders that act as scavengers feeding on the dead bodies of other captives; (2) are they captured by the plants and used as food; (3) are they adventitious residents that slowly starve to death within their prison-like walls; or (4) are they "normal" inhabitants, possibly differing in

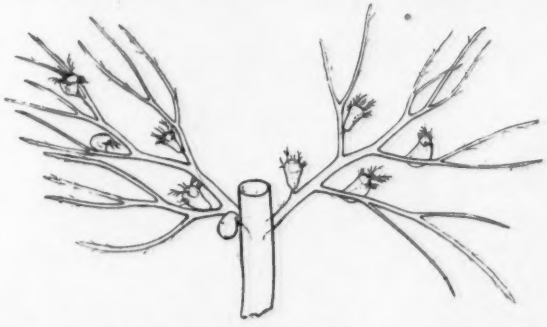


FIG. 1. Part of a branch of Utricularia showing bladders. × 4.

species from free-living protozoa, that maintain more or less intimate parasitic or symbiotic relations with the utricles. If the last named possibility is correct these protozoa may be thought of as living within a sort of primitive plant stomach the liquid contents of which consist of digestive ferments and a certain quantity of organic materials in various stages of diges-

[1] From the Department of Medical Zoölogy, School of Hygiene and Public Health, Johns Hopkins University, and the Mt. Desert Island Biological Station, Salisbury Cove, Maine. The writer is indebted to Mrs. Ethel Norris Rask for making the drawings.

FIG. 2. Side view of a normal bladder with side walls compressed. × 70.

tion. The reactions of intestinal flagellates to their environment within the digestive tract of their hosts represent a very interesting phase of the subject of host-parasite relationships which has occupied the attention of the writer for the past five years. The studies described below were undertaken, therefore, not only because of the intrinsic interest of the subject, but also because they might throw some light on the factors involved in the relations of intestinal flagellates to their entozoic environment.

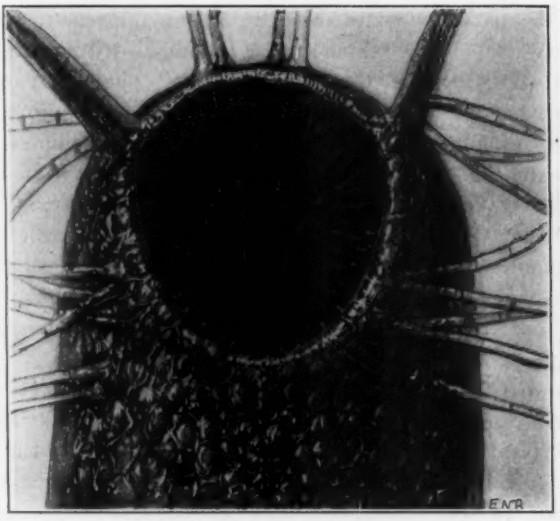


FIG. 3. View looking down into the vestibule of a bladder showing the two pairs of bristles and various glandular hairs on the valve, and other glandular hairs extending out from the sides of the vestibule. × 110.

Specimens of *Utricularia vulgaris* v. *americana* Gray live in fresh-water ponds and are often present in great abundance floating near the surface. A typical branch is shown in Fig. 1. The utricles or bladders are borne at intervals along the branches. Fig. 2 represents a side view of a bladder showing the bristles which extent out above and on either side of the mouth opening. Within the mouth is a shallow vestibule at the bottom of which is a thin, flexible valve which is attached at the top and sides but free below, where it rests tightly against an inward pro-

jecting mass of cells. This valve can open only inward. A view looking down upon the mouth opening is shown in Fig. 3. This view reveals two pairs of bristles projecting from the valve near the lower edge, a row of glandular hairs near by, and numerous glandular hairs attached to the valve near its upper edge. Extending out into the vestibule from the side walls are many glandular hairs of various lengths. The bladders vary in size from about 1 mm. to 4 mm. in length. They are usually filled with liquid but may contain bubbles of gas. They were once thought to be floats which kept the plant near the surface of the water, but the presence of small animals within them soon placed them among the "insectivorous" plants. A typical bladder when "set" and ready to function as a trap for the capture of minute aquatic animals is laterally compressed as shown in Fig. 4. Just after the capture of an organism the bladder is much distended as indicated in Fig. 5. Darwin (1875) noted that the bladders varied much in thickness according to the quantity of water contained in them and that they "are always somewhat compressed," but as will be explained later he did not realize the significance of the compressed and expanded conditions.

The remarkable ability of these bladders to capture free-swimming aquatic organisms is well indicated by an examination of a typical branch. The branch studied consisted of a main stem 110 cm. long bearing 4 side branches the combined length of which was 110 cm. The number of bladders per cm. on the main stem was estimated as 90, and on the side branches, as 36. The total approximate number of bladders was therefore 13,860. The organisms within 10 mature bladders selected at random were counted. These consisted of entomostraca, insect larvæ, rotifers, usually euglenas and often other protozoa. Entomostraca represented the principal article of food; these ranged from 6 to 22 per bladder with an average of 12. A conservative estimate of the number per bladder would be 10, which would place the total number of entomostraca captured by this part of the plant at about 150,000. The bladders live for some time and as the victims die and break down fresh specimens are captured, so that the total number captured by the plant during

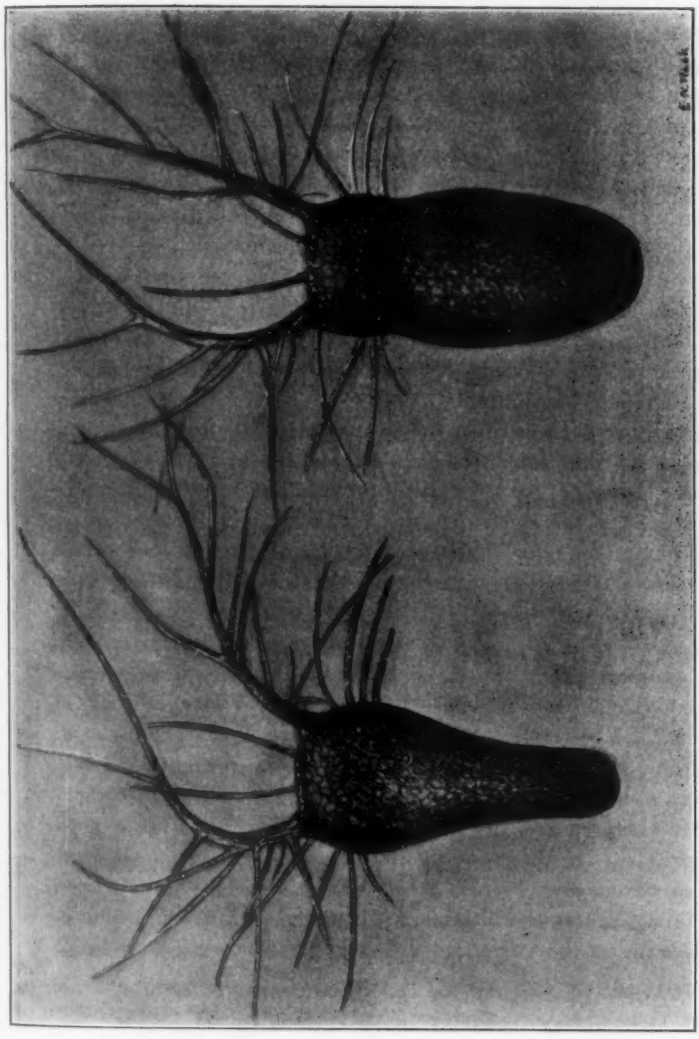


FIG. 4. Bladder compressed and ready to capture any animal that provides the proper stimulus. × 70.

FIG. 5. Bladder expanded after the capture of an organism. × 70.

the summer must be enormous and must add very materially to its nutriment if the organic material is absorbed as Darwin seems to have proved.

The protozoa that are able to live within the bladders are few both in number of species and number of individuals. Euglenas, as noted elsewhere, are rather common inhabitants. Minute ciliates and flagellates as well as small amœbæ may also be present. Darwin mentions that, "In all cases the bladders with decayed remains swarmed with living Algæ of many kinds, Infusoria, and other low organisms, which evidently lived as intruders." He does not seem to have realized that some of these Infusoria might have been captives instead of intruders.

So far as I have been able to discover the correct method of capture of organisms by the utricularia bladder has never been described.* Only after 69 of a total of 91 experiments were carried out did I discover the significance of the compressed and distended condition of the bladders, and this was finally revealed to me because of the large size of the bladders borne by the plants found near Bar Harbor, Maine. Paramecia were found to be easily and quickly captured, but many hours were spent in attempts to determine exactly how this was accomplished. The bristles around the entrance seemed rather to hinder than to guide them to the opening, since many specimens that might have entered gave the avoiding reaction and swam away when they encountered these bristles. Perhaps as Darwin suggests "their use probably is to prevent too large animals from trying to force an entrance into the bladder, thus rupturing the orifice." Only a very few of the paramecia that swam into the entrance were captured and those that were captured were taken in so rapidly that it was impossible to determine whether they forced their way in or the valve opened to receive them. Darwin says, "Animals enter the bladders by bending inwards the posterior free edge of the valve, which being highly elastic shuts again

*Since this paper was written I have had better access to literature and find that the method by which Utricularia captures its prey was more or less correctly described independently by Brocker in 1911 (Ann. de Biol. Lacustre, VI), by Ekambaram in 1916 (Agric. Jour. India) and by Withycombe in 1924 (Jour. Linn. Soc. Bot., 46). I am indebted to Mr. A. F. Skutch for the reference to Brocker's paper.

instantly." This describes only in part the method of capture. The solution of the problem was reached when a compressed bladder whose valve was touched with a needle at the lower, free edge suddenly became distended by an inrush of water. A similar reaction occurred in every compressed bladder similarly treated, but no change was evident when the valve of a distended bladder was touched with a needle. Several experiments were carried out to test the capacity of bladders to recover their compressed condition after being distended by the touch of a needle.

Experiment M. 15.—Five branches were selected and a number of bladders on each were distended by touching the lower part of the valve with a needle: branch *a*, 7 bladders; *b*, 4; *c*, 4; *d*, 4; *e*, 4. Forty minutes later these bladders had all regained a compressed condition. Several of them contained large insect larvae and many of them crustaceans in various stages of decay, but apparently the previous capture of food had no influence on their assumption of the compressed state. These bladders were all again distended as before. When reëxamined in 20 minutes all bladders on branches *a*, *b* and *c* were found to be compressed and on each of branches *c* and *d*, 2 were completely and two partially compressed. They were all again distended. Ten minutes later none of the bladders had entirely recovered. Fifteen minutes later some had entirely recovered, some almost and others only slightly. At this time the bladders were all again distended. Two hours and 15 minutes later all bladders on branches *a*, *b*, and *c* had recovered and became distended when touched with a needle, but only 2 on each of branches *c* and *d* were compressed. Two hours and 45 minutes later all bladders on branches *a*, *b*, and *c* had recovered except 2 on each which had only partly recovered; none recovered on branch *c*; and 2 recovered on branch *d*. All bladders that were entirely or partly compressed were distended with a needle at this time. Seventeen hours later or 24 hours after the beginning of the experiment, on branch *a*, 4 bladders had entirely recovered and 3 had partly recovered but became fully distended when touched with a needle; on branch *b*, 2 had entirely recovered and 2 had partly recovered, all became distended when stimulated; on branch *c*, 2 had entirely recovered, one had partly recovered and one was not compressed at all; on branch *d*, none of the experimental bladders had recovered, and it was noted that all the other bladders on this branch were distended, due possibly to some change in the physiological condition of the branch; on branch *e*, one partly recovered, the other three were fully distended.

This experiment gives some idea of the rapidity with which the bladders may capture their prey. The length of time from the capture of an organism until the trap is set for another is apparently about 20 minutes, and it is evident that the capture of organisms may occur a number of times in one day. The failure of some of the bladders to regain the compressed condition after

being distended several times may have been due in part to injury from the needle and in part to the separation of the branches from the rest of the plant. The latter seems probable at least in the case of branch *d*.

Experiment M. 22.—This experiment was carried out with 10 detached bladders, 5 from each of 2 stems. Bladders may be handled quite roughly without their becoming distended and all of these 10 were successfully detached in the compressed condition. They were then distended with a needle. Forty minutes later 8 of them had again become compressed and the remaining 2 had partially regained their compressed condition.

These results show that the processes of both distention and compression occur in detached bladders.

Compressed bladders may become distended in several ways besides that of touching the valve with a needle. When pressure is applied to either side of the walls of the entrance by squeezing the anterior end of the bladder between the arms of a fine forceps, thus opening the valve, the bladder becomes distended. Distension similarly occurs when pressure is applied antero-posteriorly. Compressed bladders may also be made to distend by puncturing one side with a needle thus allowing the entrance of liquid from outside.

The difference in the amount of liquid in compressed and distended bladders is considerable. Five branches were selected and 3 large compressed and 3 large distended bladders were detached from each, dried on the outside with filter paper and the contents withdrawn with a fine pipette. It was found that the contents of the 15 distended bladders amounted to approximately 88 per cent. more than that of the 15 compressed bladders. That is, when a bladder becomes distended it draws in a volume of the outside medium equal to about 88 per cent. of that already present. The process of distention is remarkably rapid and therefore the outside medium enters with considerable force.

The amount of suction caused by the expansion of the walls of the bladder is indicated by the following experiments. Paramecia were killed with heat and placed at various distances from the entrance; the valve was then touched with a needle. It was found that specimens as far away as 2 mm. were easily drawn into the bladder. A dead insect larva 2 mm. long was dissected out of one bladder and gently pushed against the valve

of another bladder; the entire larva was instantly drawn in, although the bladder was hardly longer than the larva. A living insect larva of the same length was placed at the entrance of another bladder; when it touched the lower edge of the valve it was also entirely and instantly drawn in. Part of a slender branch of a utricularia plant was presented to another bladder; this was drawn in until one end touched the posterior end of the bladder and the other end projected from the entrance. No more of this branch was taken in during the succeeding 24 hours.

It is evident from these experiments that the animals taken into the bladders do not try to force an entrance, but are sucked in when, by their movements, they stimulate the plant in such a way as to cause the valve to open and the walls of the bladder to expand. The exact mechanism of this process is still to be worked out.

Other species of Utricularia probably capture their prey in a similar manner. One of these *Utricularia intermedia* Hayne, which bears bladders on separate leafless branches that lie on the bottom was examined at Salisbury Cove, Maine; its bladders were compressed, but expanded when stimulated with a needle just as did those of *U. vulgaris* v. *americana*.

Various investigators have attempted to determine how utricularia bladders capture their prey. Darwin carried on experiments somewhat similar to those described above but failed. That he very nearly succeeded is evident from the following account of his experiments.

"As I felt much difficulty in understanding how such minute and weak animals, as are often captured, could force their way into the bladders, I tried many experiments to ascertain how this was effected. The free margin of the valve bends so easily that no resistance is felt when a needle or thin bristle is inserted. A thin human hair, fixed to a handle, and cut off so as to project barely $\frac{1}{4}$ of an inch, entered with some difficulty; a longer piece yielded instead of entering. On three occasions minute particles of blue glass (so as to be easily distinguished) were placed on valves whilst under water; and on trying gently to move them with a needle, they disappeared so suddenly that, not seeing what had happened, I thought that I had flirted them off; but on examining the bladders, they were found safely enclosed. The same thing occurred to my son, who placed little cubes of green box-wood (about 1/60 of an inch, .423 mm.) on some valves; and thrice in the act of placing them on, or whilst gently moving them to another spot, the valve suddenly opened and they were engulfed. To ascertain whether the valves were endowed with irritability, the surfaces of several were scratched with a needle or

brushed with a fine camel-hair brush, so as to imitate the crawling movement of small crustaceans, but the valve did not open. We may, therefore, conclude that animals enter merely by forcing their way through the slit-like orifice; their heads serving as a wedge."

Mrs. Treat (1875) seems to have been the first to study the method of capture employed by the utricularia bladders. Her description, in part, is as follows: "They (little animals) would sometimes dally about the open entrance (the vestibule) for a short time, but would sooner or later venture in, and easily open or push apart the closed entrance at the other extremity. As soon as the animal was fairly in, the forced entrance closed, making it a secure prisoner." "I never saw even the smallest animalcule escape after it was once fairly inside the bladder."

According to Büsgen (1888) animals do not force their way into the bladder between the edge of the valve and the wall of the bladder as supposed by Darwin. They were able to creep about on the outer surface of the valve for considerable periods without being engulfed but suddenly the valve would open and close again and the animals would disappear within. He believed that the mucus on the wall of the bladder where it meets the valve helps to precipitate the prey into the cavity of the bladder and that the mucus and bristles at the mouth of the bladder may stimulate the animal to violent movements which cause the valve to open. The sudden opening of the valve led him to think that he was dealing with a phenomenon of irritability, but he found as did Darwin that stimulation of the bristles with a needle or brush did not cause the opening of the valve. Büsgen almost arrived at the correct method employed by the bladders in capturing animals but apparently did not note the distension of the walls of the bladder and hence missed the essential feature of the process.

Goebel (1891) agreed with Mrs. Treat and with Darwin that the captured animals force their way into the bladders. Certain of the glandular hairs were supposed by him to be attractive to microörganisms, and other glandular hairs to be so located as to aid the animals to enter the bladder.

Mueller (1925) in an article entitled "The Rotifer Group" published two figures showing utricularia bladders. The legends of these figures read in part as follows: (1) "If a rotifer or other

tiny pond animal chances to rest upon this door, it immediately slips through the slot which quickly closes upon the prisoner. The animal swims about inside the utricle and finally dies." (2) "Larva of harlequin fly (*Chironomus*) just captured by plant. This larva was feeding upon food particles adhering to the branched spines projecting from the vesicle, and gradually worked its way down the stem until it touched the slippery trapdoor which straightway opened and caught it by the head. Because of the downward-pointing hairs lining the vesicles the struggles of the larva merely draw it further into the trap." The method of capture described in these legends does not agree with that I have found to be true of the species I have studied. Apparently the legends were written without any actual observations of the process. Furthermore I know of no "downward pointing hairs lining the vesicles" that might draw an insect larva into the bladder.

The most recent papers on the capture of organisms by utricularia are by Brumpt (1925) and Langeron (1925). Brumpt notes the capture of mosquito larvæ by the bladders and stresses their importance in the control of malaria by mosquito reduction but does not discuss the method of capture. Langeron states that animals penetrate easily the bladders of both aquatic and terrestrial species of Utricularia and that only a slight pressure on the valve is sufficient to precipitate them into the cavity, but does not describe the process further.

Organisms once captured appear never to escape. Frequently, as Darwin noted, insect larvæ are sometimes too long to be drawn in at one time, and bladders are to be found with part of the body of the larva inside and the rest projecting from the entrance. Brumpt (1925) has shown that mosquito larvæ are often drawn in tail first and on account of their large size are usually to be found with their heads extending out from the entrance. Brumpt accounts for this by the habit of certain insect larvæ of inserting the end of the tail into crevices. In this way he supposed that insect larvæ forced their way into the bladders. A more simple explanation, now that the method of capture is known, is that the ventral brush of the anal segment which the mosquito larva moves briskly in swimming stimulates the bladder to action and that it is therefore the posterior end that is drawn in first.

As already noted, a distended bladder regains its compressed condition in about 20 minutes. How this is accomplished by the plant I do not know but it involves the absorption by or expulsion from the bladder of about 88 per cent. of its liquid contents. Several experiments were performed in an attempt to determine whether liquid passes into and out of the bladders at any time except when organisms are captured.

Experiment 66.—Seven branches bearing from 6 to 19 bladders each were immersed for 2½ hours in aqueous solutions of carmine, india ink, eosin, safranin, and crystal violet. All bladders were deeply stained in safranin and crystal violet. The walls were not stained in the other solutions but many of the bladders contained the coloring matter; 4 of 9 bladders in carmine contained red granules; 8 of 14 in eosin were filled with this solution; and 10 of 15 took in India ink.

Experiment 67.—Branches bearing 100 bladders were immersed in an aqueous solution of eosin for 3 hours. The solution was found within 69 of these bladders at the end of this period, mostly in young and medium sized specimens.

Experiment 68.—Branches bearing 52 bladders were immersed in a paramecium culture for 2 hours and then in an eosin solution for 2½ hours. Paramecia were captured by 12 bladders and became stained with eosin in 11 of them. Nine of the bladders that did not capture paramecia took in eosin and 32 did not.

Experiment 69.—Nine branches bearing 79 bladders were immersed in a paramecium culture 2 hours and then in an eosin solution 2 hours. Paramecia were captured by 67 bladders and became stained with eosin in 65 of them. The 12 bladders that did not capture paramecia all took in eosin.

Experiment M. 20.—Compressed bladders were placed in an eosin solution and expanded thus adding this solution to their contents. They were then washed in water, placed in hollow ground slides and examined. None of these bladders recovered the compressed condition.

These experiments prove that solutions of eosin, carmine and India ink are taken in by bladders that have not captured paramecia and eosin solution by bladders that have captured paramecia. That this is due to spontaneous expansion of the bladders is possible but not probable. How the solutions were taken in is still in doubt. This is true also regarding the passage of liquid out of the bladders. This is a problem for the plant physiologists to solve, and work on it is now in progress.

After discussing the probable function of the glands located around the exterior of the entrance Darwin concludes that these "are adapted to absorb matter from the putrid water, which will occasionally escape from bladders including decayed animals." He remarks, however, that, "The valve fits so closely, judging from the result of immersing uninjured bladders in various

solutions, that it is doubtful whether any putrid fluid habitually passes outward. But we must remember that a bladder generally captures several animals, and that each time a fresh animal enters, a puff of foul water must pass out and bathe the glands." On the contrary, my experiments prove that when an animal enters a bladder, medium is taken in rather than forced out, and that fluid passes outwards between the time of one capture and that of the next succeeding capture, *i.e.*, during the period of compression. Darwin's main conclusion may, therefore, be correct, provided the glands around the entrance absorb material if this is forced out of the valve during the compression of the bladder.

Experiments with Euglenas.

Euglenas are of frequent occurrence in the bladders of Utricularia plants in nature. In 1922 euglenas were found to be present in almost all of the bladders of Utricularia plants obtained from a pond on the campus of the Johns Hopkins University and almost every bladder was infected on plants that had been kept in a dish in the laboratory for several months. The number present in a single bladder as determined by counting those in ten bladders selected at random, ranged from 8 to 510, with an average per bladder of 215 (Hegner, 1923). The pond from which these plants were taken contains large numbers of euglenas at certain times of the year and these bladders may have become infected with these free-living specimens. It was believed, however, at that time, that the utricularia bladders contained digestive ferments and on this account might successfully withstand the conditions within the digestive tract of tadpoles in which euglenas had been proved to be normal inhabitants (Hegner, 1923, Wenrich, 1923). When fed to tadpoles, however, these euglenas were promptly digested. Euglenas were also noted in many of the bladders on plants of *Utricularia vulgaris* var. *americana* collected near Bar Harbor, Me., although not in as great numbers as in those on plants collected in Baltimore.

Utricularia bladders lose their infection with euglenas when kept for a long period in the laboratory. Bladders of Utricularia were used for experimental purposes in the spring of 1925 that were taken from plants that had been kept in large stender

dishes in the laboratory for about eight months. These bladders were almost entirely free from euglenas although when first brought into the laboratory the bladders were infected with large numbers. This may be accounted for as follows. Bladders do not live very long, but gradually loose their chlorophyll, break away from the plant and drop to the bottom. Here they disintegrate in course of time thus liberating any euglenas contained in them. These euglenas either live in the water or are captured by the new bladders that are continually developing. Their habitat within the bladders is very restricted and their escape from the old bladders and reinfection of new bladders difficult. Probably for these reasons, the number of euglenas decreased in plants that were kept under laboratory conditions.

Free-living euglenas are captured by bladders and live normally and multiply within bladders. Bladders were immersed in cultures containing many free-living euglenas or euglenas were injected into them with a fine pipette.

Experiment 2.—One of ten detached bladders became infected in 24 hours with one active free-living euglena.

Experiment 4.—Thirteen attached bladders failed to become infected in 48 hours.

Experiment 23.—Eleven of twenty detached bladders became infected in 40 hours. The numbers present in these bladders were as follows: 3 bladders with one each; 3 with 2 each; one with 3; and 4 with 6 each. The average number per bladder was 3.3. These bladders were examined at intervals of 3 days for 9 days. They all remained infected during this period. At the end of 3 days the number had increased in 6 of the bladders; the average per bladder increased from 3.3 to 6.7. A further increase took place during the next three days, the average rising from 6.7 to 15.1. During the succeeding three days this average increased from 15.1 to 20.1 per bladder. The numbers present in the individual bladders at this time were 50, 40, 30, 30, 16, 10, 8, 7, 6 and 4. One infected bladder had broken down and allowed the euglenas to escape.

Experiment 20.—Euglenas were injected with a fine pipette into 10 bladders attached to 4 stems, each bladder receiving from one to eight specimens. These were examined on 7 days during the succeeding period of 9 days. The number increased in 7 of the ten bladders; the other 3 bladders became detached before the observations were completed.

These experiments show that bladders may become infected with free-living euglenas and that these not only are not destroyed within the bladders but appear to move about normally and reproduce. Normal activity and reproduction also occur in attached bladders into which euglenas have been injected. These

results suggest that the euglenas found in bladders in nature are free-living species that were captured by the bladders and were unable to escape and that these euglenas are either able to withstand any digestive ferments contained in the bladders or do not stimulate the production of such ferments by the bladder cells.

Euglenas are not injured in bladders that have captured and killed paramecia.

Experiment 30.—Thirty-nine bladders attached to 4 stems were immersed in a euglena culture for three hours and then in a paramecium culture for one hour. Seventeen of the bladders became infected with paramecia, from one to 17 in each bladder, the average per bladder being 3.6. Four of the bladders became infected with one paramecia each and three of these also contained euglenas, one with 5 and 2 with 2 each. In every case the paramecia had been killed but the euglenas were active.

Experiment 33.—Seventeen bladders attached to 2 stems were immersed in a paramecium culture for 2½ hours; 13 became infected with paramecium as follows: 5 with one each, 5 with 2, one with 4, one with 6, and one with 8. After an interval of 4 hours when all the paramecia had been killed and many had already disintegrated euglenas were injected with a capillary pipette into each of the infected bladders. Nineteen hours later the euglenas within these bladders were active and apparently normal.

Experiment 51.—Forty-four bladders attached to 6 stems were immersed in a mixed culture of paramecia and euglenas and allowed to remain for 3 hours. Twenty-one bladders became infected with paramecia as follows: 18 with one each, 2 with 2, and one with 3; twenty-nine bladders became infected with euglenas; and 14 became infected with both. These bladders were examined daily during the four succeeding days and active euglenas were observed in 13 of the 14 bladders that captured paramecia. In the 14th bladder the euglenas were not moving. The euglenas were active in 14 of the bladders that had not captured paramecia and quiet in the 15th. Seven bladders captured paramecia but no euglenas and 6 bladders did not become infected with either.

Experiment 50.—In this experiment, which was initiated for another purpose, one bladder was found to contain active euglenas in the presence of disintegrating paramecia.

In the above experiments paramecia and euglenas became associated within the bladders of Utricularia in three different ways: (1) bladders were first infected with euglenas and then with paramecia, (2) bladders were first infected with paramecia and then euglenas were injected into them, and (3) bladders became infected with both in a mixed culture of paramecia and euglenas. The results show that regardless of the method of infection the euglenas remain active in bladders that have brought

about the death and disintegration of paramecia, and that any digestive ferments that might have been produced by the bladder stimulated by the presence of paramecia, are not injurious to the euglenas.

Liquid from bladders which have captured and killed paramecia does not kill euglenas.—In Experiment 36, the liquid was squeezed out of 25 bladders that had captured and killed paramecia. Euglenas added to this liquid were still active 18 hours later.

Experiments with Paramecia.

The bladders of utricularia capture and kill paramecia. A preliminary experiment proved that paramecia are readily captured and killed by utricularia bladders.

Experiment 1.—A branch bearing 14 bladders was immersed in a culture containing large numbers of paramecia. When examined on the following day, 22 hours later, all bladders were found to contain paramecia only one of which was alive. It was evident that a number of specimens had disintegrated so the exact number captured could not be determined, but there were at least 4 bladders with one specimen each, 3 with 2, 5 with 3, and 2 with 5. The specimen that was still alive died within one hour and was beginning to disintegrate 2 hours later. On the next day, 48 hours after the experiment was begun, the only traces of paramecia remaining in the bladders, which had in the meantime been taken out of the paramecium culture, were fine granular masses representing thoroughly disintegrated paramecia. It is evident from this experiment that protozoa as small as paramecium are captured by the bladders and are killed and disintegrate within a few hours.

Paramecia are captured by either bladders that are attached to or detached from the utricularia plant in as short a time as 8 minutes. Branches bearing bladders were placed in paramecium cultures or bladders were detached from the plant and immersed. They were taken out; washed in water, and examined at various intervals. The following data were obtained from 8 experiments.

Total number of bladders	180
Total number positive for paramecia	56 or 30%
Total number negative for paramecia	124 or 70%
Number of bladders attached to plant	150
Number of attached bladders positive for paramecia	47 or 30%
Number of detached bladders	30
Number of detached bladders positive for paramecia	9 or 30%

Time required to capture paramecia, from 8 minutes to 2 hours; average 56 minutes.

Time required for attached bladders to capture paramecium, from 30 minutes to 1½ hours; average, 56 minutes.

Time required for detached bladders to capture paramecia, from 8 minutes to 2 hours; average, 59 minutes.

Number captured per positive attached bladders, from one to 6; average, 1.54.

Number captured per positive detached bladder, from one to 4; average, 1.56.

These data indicate that no difference exists between attached and detached bladders so far as the capture of paramecia is concerned. Regarding the length of time required for the capture of paramecia the data are not accurate since the bladders could not be kept under continuous observation; but in a culture containing active paramecia about 30 per cent. of the bladders succeeded in capturing one or more paramecia within one hour.

The number of paramecia captured depends on the character of the culture medium. Six experiments were carried out to test the effects of different culture media on the capture of paramecia. It was thought that the bladders might be stimulated to greater activity by media containing nutrient materials. The contrasting media used in these experiments were as follows (in every case the number of paramecia in the medium was increased by centrifuging the hay infusion, and the bladders were attached to branches of the plant): Exp. 29, spring water v.s. hay infusion; Exp. 38, tap water v.s. hay infusion v.s. beef meal v.s. malted milk v.s. 0.7% saline; and Exp. 41, 42, 44 and 46, hay infusion v.s. tap water after washing in tap water.

Experiment 29.—In hay infusion, 20 of 69 bladders, or 29 per cent., captured 35 specimens in a period of 2 hours; the number per positive bladder ranged from one to 86, and the average per positive bladder was 1.75. In spring water, 62 of 73 bladders, or 85 per cent., captured 272 specimens in the same period; number per positive bladder, one to 19; average, 4.40.

Experiment 38.—In hay infusion, 13 of 40 bladders, or 32 per cent., captured 23 specimens or 1.8 per positive bladder.

In tap water, 5 of 47 bladders, or 11 per cent., captured 5 specimens or 1 per positive bladder.

In 0.5 per cent. beef meal solution, 17 of 44 bladders, or 39 per cent. captured 21 specimens or 1.2 per positive bladder.

In 1.0 per cent. malted milk solution, 16 of 44 bladders, or 36 per cent., captured 27 specimens or 1.7 per positive bladder.

In 0.7 per cent. saline solution, 2 of 43 bladders, or 5 per cent., captured 2 specimens or 1.0 per positive bladder.

Experiments 41–46.—In hay infusion, 9 of 30 bladders captured 10 specimens; 4 of 30 captured 4; 19 of 61 captured 24; and 8 of 30 captured 10, respectively. Totals: 40 of 151 bladders captured 48 specimens; 26 per cent. of the bladders captured an average of 1.2 specimens per bladder.

In tap water, 12 of 30 bladders captured 16 specimens; 10 of 30 bladders captured 12; 26 of 66 captured 31; and 6 of 30 captured 8, respectively. Totals: 54 of 156 bladders captured 67 specimens; 35 per cent. of the bladders captured an average of 1.3 specimens per bladder.

From these results tap water seems to be a more effective medium for the capture of paramecia than hay infusion; 44 per cent. or 121 of 276 bladders captured 344 or 2.8 per bladder in tap water, whereas only 28 per cent. or 73 of 260 bladders captured 1.5 per bladder in hay infusion. The other media used were not sufficiently studied to give significant results, but saline solution is apparently disadvantageous, and beef meal solution and malted milk solution are as favorable as hay infusion or even more favorable, but not as effective as tap water. Observations of the activities of the paramecia in different media revealed the fact that the organisms swam about more rapidly in tap water than in any other medium. This means that in tap water the chances that paramecia will come in contact with the valves of the bladders more frequently and with more force are greater than in the other media and would lead to a larger number being captured in a given period. The conclusion based on these experiments is therefore that the differences in the number of specimens captured are due to the effects of the media on the rate of speed of the paramecia rather than their effect on the bladders.

Paramecia are killed by bladders, on the average, in a period of about 75 minutes. That paramecia die after being captured by utricularia bladders was proven by Exp. 1 described above. Considerable effort was made to determine the exact length of time required to bring about their death. Data from two types of experiments are available. In 16 experiments stems bearing bladders were placed in cultures containing paramecia and removed usually at the end of 2 hours but sometimes in one hour or in 4 hours. An immediate examination provided data regarding the number captured during this period and the numbers that had been killed or were still alive. The total number captured in these 16 experiments was 780 of which 563 were dead and 217 were alive when the bladders were examined. A summary of the data is as follows:

9 of 21 or 43 per cent. were dead one hour after the beginning of the experiments.
12 of 21 or 57 per cent. were alive one hour after the beginning of the experiments.
416 of 603 or 69 per cent. were dead two hours after the beginning of the experiments.
187 of 603 or 31 per cent. were alive two hours after the beginning of the experiments.
138 of 156 or 88 per cent. were dead four hours after the beginning of the experiments.
18 of 156 or 12 per cent. were alive four hours after the beginning of the experiments.

These data show that the percentage of paramecia killed increases from 43 one hour after the beginning of the experiments to 88 four hours after the beginning of the experiments. The exact length of time necessary to kill these protozoa could not of course be determined in this way.

Three other experiments were carried out in the following manner. Branches bearing bladders were immersed in a paramecium culture for a short period, then washed, placed under a large cover glass in a petri dish and flooded with water. Examinations of these preparations were made at frequent intervals.

Experiment 50.—A record of 9 captured paramecia shows one dead in 45 minutes; 2 active at least 42 minutes but dead in 56 minutes; one alive 49 min., but dead in 61 min.; one alive 54 min., but dead in 72 min.; one alive 64 min., but dead in 73 min.; one alive 75 min., but dead in 91 min.; one alive 84 min., but dead in 103 min.; one alive 104 min., but dead in 114 min. The average period during which these paramecia continued to live was 64 minutes and the average time until their death was 75 minutes.

Experiment 53.—In this experiment a record was kept of 23 captured paramecia. The period that these remained active after capture ranged from 20 to 127 minutes, with an average of 48 minutes, and the period until their death ranged from 34 to 141 minutes or an average of 72 minutes.

Experiment 54.—A record was made of 62 specimens in this experiment. Two were active 20 minutes; 3 from 30 to 40 min.; 5 from 40 to 50; 9 from 50 to 60; 15 from 60 to 70; 3 from 70 to 80; 9 from 80 to 90; 4 from 90 to 100; 3 from 100 to 110; and 9 from 113 to 170. Two were dead in from 20 to 30 minutes; 2 from 40 to 50 min.; 5 from 50 to 60; 7 from 60 to 70; 15 from 70 to 80; 5 from 80 to 90; 7 from 90 to 100; 2 from 100 to 110; 5 from 110 to 120; and 10 from 123 to 180. The active period of the greatest number of specimens was from 50 to 80 minutes, 35 of the 62 falling within this range. The average active period was 78 minutes. The death period of most of the specimens was from 50 to 98 minutes, 39 of the 62 falling within this range. The average death period was 93 minutes.

The average active period of the paramecia studied in Exps. 50, 53 and 54 was 64 minutes for 9 specimens; 48 minutes for 23; and 78 minutes for 62. If the data of these three experiments

are combined the average active period after capture is found to be 69 minutes. The average period until death in the same experiments was 75 minutes for 9 specimens; 72 minutes for 23; and 93 minutes for 62. When the data of the experiments are combined the average period of the 94 spcimeens studied is found to be 85 minutes. It may be concluded that the actual death period lies between that when the specimens were last observed alive and that when they were first observed to be dead, which is on the average between 69 and 85 minutes. Seventy-five minutes may, therefore, be selected as approximately the length of time paramecia, on an average, remain alive after being captured by the bladders of utricularia.

When injected into attached bladders paramecia may die within a short period or remain alive for many days. In three experiments paramecia were injected with a fine pipette into bladders attached to branches of the utricularia plant.

Experiment 13.—Paramecia were injected into 6 bladders attached to one stem, from 2 to 10 specimens in each bladder. All were alive at the end of 2½ hours, but were dead in 3 of the bladders at the end of 26 hours. The paramecia remained alive in one bladder for 6 days and escaped probably because of the disintegration of the wall of the bladder. In another bladder two of the original 5 paramecia were still alive on the 16th day, but were very sluggish and quite transparent being evidently in a starved condition. One of these became abnormal in shape during its confinement; this specimen was still present after 17 days but was gone (escaped?) on the 18th day.

Experiment 52.—Nineteen bladders attached to 6 branches were injected with from one to 11 paramecia each. In three bladders the organisms died within 26, 67 and 74 minutes respectively. In the other 16 bladders all the specimens remained alive at least 2 hours and in some of these the paramecia lived for 4, 5, 6 or 7 days.

Experiment 57.—Fifty-three bladders attached to 7 stems were injected with from one to 20 paramecia each. They were killed in 32 of the bladders within 4 hours as follows: in 3 bladders in 25, 51 and 55 minutes respectively; in 8 bladders within from one to 2 hours; in 11 bladders in from 2 to 3 hours; and in 10 bladders in from 3 to 4 hours. The paramecia disappeared (escaped?) from 5 bladders within 24 hours, but remained active in the remaining 16 bladders at least one day, and in 3 of them 2 days, in 3 of them 3 days, and in one of them 5 days.

When the results of these injection experiments are compared with those described in which the paramecia were captured by the bladders the most noticeable difference is the greater length of time required for the death of most of the organisms, extending as in Exp. 13 to a period of 17 days. This suggests that many

of the injected bladders were not capable of bringing about the death of the paramecia and might not have captured specimens if placed in a culture medium containing them, or that the condition of the bladders was modified in some way during the process of injection. This subject will be referred to later.

When injected into detached bladders paramecia may die within a short period or remain alive for many days. Three experiments furnish data regarding the death of paramecia injected into detached bladders.

Experiment 11.—From 2 to 12 paramecia were injected into 4 bladders. They died in two bladders within 18 hours; escaped from one; and 9 specimens remained alive in the remaining bladder for 14 days; these paramecia were evidently suffering from lack of food. One of them remained alive for 16 days, but was gone (escaped?) on the 17th day.

Experiment M. 6.—Six bladders were injected with from one to 6 paramecia each. All were alive at the end of 1½ hours; all died within 6 hours except those in one bladder which died within 22 hours.

Experiment M. 12.—Fifteen bladders were injected with paramecia. They died in 10 bladders within 2 hours; in the other five they lived at least 2 days in 2, 3 days in 2, and 5 days in one.

These results are similar to those obtained by injecting paramecia into attached bladders and indicate that the separation of bladders from the plant has no effect upon their relation to the prey they capture.

Paramecia captured by or injected into attached bladders that have been irrigated with water usually die within several hours or live for several days. The bladders were irrigated by first inserting a fine pipette through the entrance and sucking out the contents. Further suction was then applied which drew a continuous stream of water into the bladder from the outside and up into the pipette. The inside of the bladders was thus thoroughly washed out and the bladders filled with fresh water.

Experiment 58.—Thirty-eight bladders on 5 branches were irrigated, placed in a paramecium culture for 1½ hours and then examined. Four bladders captured paramecia. Bladder *a* contained 2 dead and one alive; *b*, 2 alive; *c*, 4 dead and 4 alive; *d*, one alive.

Experiment 62.—Thirty-four bladders on 5 branches were irrigated, placed in a paramecium culture for 2 hours and then examined. Five bladders were positive. The paramecia escaped from 2 of the bladders; they were dead in 2 and alive in one.

Experiment 63.—Only one of 39 bladders on 7 branches became infected with paramecia; two specimens in this bladder died within 2 hours.

Experiment 64.—Only-one of 31 bladders on 5 branches became infected; the two specimens in this bladder died within 2 hours.

Experiment M. 11.—Six bladders on each of 4 branches were irrigated and then inoculated with paramecia. All were alive at the end of 1½ hours. After 9 hours the paramecia were dead in 19 bladders, alive in 4, and had escaped through a break in the wall of one. At the end of 24 hours the paramecia were dead in 22 and alive in one bladder.

Experiment M. 13.—Six bladders on each of 4 branches were irrigated and then inoculated with paramecia. All were alive after 2½ hours. At the end of 4½ hours all were alive in the bladders on branch *d*, all were dead on branch *b*, and 5 were dead and one alive on branches *a* and *c*.

Experiment M. 17.—Four branches bearing 6 bladders each were used. Bladders 1, 2, and 3 on each branch were irrigated and bladders 2, 4, and 6 were not; all were inoculated with paramecia. All were alive at the end of one hour. After 2½ hours all were dead on branch *b*; all were alive on branch *d*; all were dead in bladders 1, 3, 4 and 5 of branch *a* and in 4 and 6 of branch *c*. Paramecia remained alive for 54 hours in bladder 6 of branch *a*, in bladders 1, 2, and 3 of branch *c*, and in bladders 1, 3, and 5 of branch *d*. These all died within the next 4 days.

These experiments indicate that irrigated bladders do not succeed well in capturing paramecia but that the specimens captured usually die within 2 hours. Their failure to capture paramecia is due probably to the fact that many irrigated bladders do not take on the compressed condition necessary for capturing their prey (see page 243). Exps. M. 11 and M. 13 prove that paramecia inoculated into irrigated bladders usually live for several hours but that many of them die within from 4½ to 9 hours. Differences in the physiological condition of the different branches seems to be responsible for the fact that the paramecia die in all the bladders on one branch but remain alive in all those on another, as in Exp. M. 13 branches *d* and *b*. In Exp. M. 17 a critical experiment of irrigated and non-irrigated bladders is made. Very little difference is noticeable at the end of 2½ hours at which time the paramecia were dead in 6 irrigated and in only 2 non-irrigated bladders. The data indicate that irrigation delays but does not prevent the death of the paramecia.

Paramecia break down into a granular mass in attached or detached bladders that have captured them or into which they have been inoculated either with or without irrigation. The paramecia that die in the bladders of utricularia undergo disintegration in several different ways. Some of them dissolve into a mass of granules almost at once, spreading out as though an explosion

had occurred within; others become opaque and abnormal in shape and then begin to break down at one or two points usually on the side; and still others although opaque and abnormal in shape retain their identity.

Experiment 52.—The paramecia in this experiment disintegrated into a mass of granules in the attached bladders that had been inoculated with them within 26 minutes, 24 hours, 2 days, 4 days and 5 days respectively.

Experiment 53.—The paramecia became granular in attached bladders that had captured them within the following number of minutes: 38, 68, 70, 83, 85, 123, 124, 127, 194. In at least 9 bladders they remained intact for 18 hours.

Experiment 54.—The paramecia became granular in attached bladders that had captured them within the following number of minutes: 27, 70, 71, 125. Five became granular within 2½ hours, and 8 within 20 hours.

Experiment 55.—Two specimens became granular in attached bladders that had captured them within 2 hours, 2 within 2¼ hours, and 12 within 2½ hours. Fifty-five were still intact at the end of 2½ hours.

Experiment 57.—The paramecia became granular in attached bladders that had been inoculated with them as follows: in 10 bladders within 18 hours; in 16 bladders within 24 hours; and in 8 within 48 hours.

Experiment 56.—The paramecia became granular in detached bladders that had captured them as follows: 6 within 40 to 58 minutes; 12 within 64 to 95 minutes; and 2 within 2½ hours. One was still alive and 143 were dead but not granular at the end of 4 hours.

Experiment 58.—Paramecia in this experiment were captured by detached irrigated bladders. Seven hours later 3 specimens had become granular, 11 were dead but not yet broken down and one was still active.

Experiment M. 13.—Paramecia were inoculated into attached irrigated bladders. No accurate record was kept but many specimens were granular at the end of 5 hours.

This series of experiments indicate that paramecia break down into a mass of granules under various conditions, (1) in attached bladders that have captured them, (2) in detached bladders that have captured them, (3) in attached bladders inoculated with them, (4) in attached irrigated bladders inoculated with them, and (5) in detached irrigated bladders that have captured them. The principal differences seem to be the length of time required to reach the granular state and the comparative number that become granular within a brief period, *e.g.*, within one hour or 2½ hours. A larger number of paramecia became granular in a shorter length of time within bladders that were attached or detached and had captured them (Exps. 53, 54, 55, 56) than in bladders into which they had been inoculated. No differences are apparent between attached and detached bladders. From

the data available there appears to be no striking difference between bladders that have been inoculated with paramecia, attached or detached, irrigated or not irrigated.

Paramecia are captured more frequently by older, larger bladders than by younger, smaller bladders. In some of the earlier experiments the younger, smaller bladders seemed to become more readily infected with paramecia than those older and larger. Three experiments were carried out to test this. Branches bearing both young and old bladders were selected and the sections with the young and those with the old bladders cut out and placed in different paramecia cultures for 2 hours.

Experiment 39.—Three of 48 young and 6 of 48 old bladders became infected.

Experiment 40.—Eight of 30 young and 17 of 30 old bladders became infected.

Experiment 43.—Thirteen of 30 young and 11 of 30 old bladders became infected.

A total of 24 young and 34 old bladders became infected in these experiments. This is probably too small a number to be significant, but could be explained by the greater chance the older bladders offer because of the larger size of the entrance.

Paramecia do not die in a short time in old dead bladders or in mature bladders that have been killed by heat. When bladders become very old they loose their chlorophyll, become detached from the branches and fall to the bottom. Two experiments were carried out to determine whether paramecia are captured by these old, dead bladders.

Experiment 49.—Forty old bladders were placed in a paramecium culture for 2 hours. Seven of them captured specimens; none of these died and all had escaped within 24 hours, indicating that they had entered through openings in the walls of the bladders.

Experiment 63.—Sixty old bladders were similarly tested. No paramecia were captured.

Two experiments were carried out with mature bladders which were killed by being subjected to boiling water for 2 minutes.

Experiment 59.—Seven branches bearing 68 bladders were immersed in a paramecium culture for 1½ hours. All bladders were negative.

Experiment 61.—Nineteen bladders on 5 branches were inoculated with paramecia and kept under continuous observation for 2½ hours. None died. Twenty-four hours later many had escaped.

No differences in the behavior of paramecia were noted when placed in liquid from non-infected and infected bladders. Eleven experiments were carried out to determine whether any differences as regards the effect upon paramecium could be detected between the liquid from unfed bladders and that from bladders that had recently captured and killed paramecia. Liquid from the latter, as noted above (p. 254) has no effect on euglenas added to it.

Experiments 31, M. 8, M. 9, M. 14.—In these experiments the liquid from 107 bladders that had not captured paramecia was either squeezed out or withdrawn with a fine pipette, and either placed in a hollow ground slide in a moist chamber or treated as a hanging drop. Several paramecia were added to each and the preparations examined at frequent intervals. In Experiments M. 8 and M. 14 the paramecia were normally active at the end of 48 hours when the experiments were discontinued; in the other experiments the paramecia lived at least 5½ hours and death was later due to drying or to insufficient medium.

Experiments 26, 32, 60, M. 10 and M. 16.—Liquid from 203 control bladders and from 203 bladders that had killed paramecia was squeezed out or withdrawn with a fine pipette and its effects on paramecia tested. No significant difference was noted between the behavior of the paramecia in the liquid from non-infected bladders and in that from infected bladders. When a small amount of liquid was used the paramecia died in all cases within a few hours; when a larger amount was used no change was noted within 48 hours and the experiments were therefore discontinued.

The results of the experiments on paramecia described above are conclusive so far as they concern the effects of their capture or injection into the bladders under various conditions. Just how the results noted are brought about has not been determined and evidently will require for its solution extended experimental research. Among the questions that arise are the following:

What differences are responsible for the death of certain protozoa such as paramecium and stentor (see below) and the apparent ability of others such as euglena, heteronema, and phacus to live indefinitely within the bladders? (see below).

What is responsible for the rapid death of paramecia in certain bladders and their ability to live for many days in others?

Does the plant, when in a certain physiological state, secrete something into the bladder that brings about the death of paramecia?

Why do some paramecia disintegrate almost immediately and others from several hours to several days after death?

When several paramecia are confined in a single bladder, why do some disintegrate before others, as sometimes happens?

Is the same factor that is responsible for the death of paramecium also responsible for its disintegration?

Does the plant secrete something into the bladder that digests the paramecia?

It would be unprofitable to discuss these questions on the basis of the data available but certain observations not emphasized above have a bearing on them. In the first place, it is evident that paramecia are not killed quickly by confinement, since in some of the experiments specimens lived for several weeks in the bladders. The physiological state of the plant apparently has a large influence on the paramecia. For example, in Experiment 57 all the paramecia injected into bladders on one stem died within a few hours and all injected into those on another stem were all alive at the end of 24 hours. In another case (Experiment M. 13) paramecia were injected into 6 irrigated bladders on each of 4 branches; they were all dead in the bladders on three of the branches within 5 hours but remained alive in certain of the bladders on the fourth branch for 8 days. These experiments indicate that the plant when in the proper physiological condition secretes something into the bladders that kills paramecia but at other times simply confines the organisms within the bladders. Attempts were made to determine whether or not the plant exhibited evidences of hunger and satiety by examining bladders continuously over a period of several days, but the results are not significant. Another problem considered was the possible selection of organisms by the bladders. No experimental data are available on this point but the observations on the types of organisms captured and on the method of capture led to the conclusion that any organism may be captured that is small enough to enter the vestibule of the bladder and actively moves about within the vestibule.

The best tests for the presence of digestive enzymes within the bladder have been reported by von Luetzelburg (1910) who comes to the conclusion that the organisms captured by the bladders are utilized as food by the plant by means of an enzyme acting in an alkaline medium. An admixture of acid (benzoic acid) prevents the organisms from injuring the walls of the bladder. Digestion within the bladder is slow but thorough.

The valve of the bladder is closed so tightly that nothing within can escape.

At present there is little doubt regarding the utilization by the plant of material derived from the animals captured and digested. Darwin concluded from a study of the quadrifid cells in the inner walls of the bladders before and after the capture and disintegration of food that the plant absorbed nutriment derived from the captured animals. Further evidence that this is true is offered by Büsgen (1888) who measured fed and unfed plants at intervals during a period of about three weeks. The fed plants grew twice as fast as the unfed plants during this period, apparently because of the nutriment supplied by the animals captured and absorbed by the bladders of the latter.

Experiments with Other Protozoa.

Although the principal experiments were carried out with paramecia and euglenas a few other protozoa were used as opportunity offered. These included representatives of all three classes of the free-living Protozoa—Sarcodina, Mastigophora and Infusoria.

Centropyxis aculeata is captured by bladders and starved to death. Shells of specimens of this species were observed in bladders brought into the laboratory, but these apparently contained no living animals. Two experiments were performed to determine the relations of this species to the plant.

Experiment 37.—Fifteen detached bladders were placed in a concavity in water containing a number of large specimens of *C. aculeata*. Six days later 6 of these bladders were found to be infected, 4 with one and 2 with 2 each. The shells contained very little protoplasm—approximately one tenth of the original amount—but this was still alive and able to form pseudopodia. Apparently the conditions in the bladder have no directly injurious effect upon these rhizopods but deprive them of opportunities for capturing food and hence lead to their starvation.

Experiment 27.—Four bladders on one stem were inoculated, three with one and one with 2 specimens of *C. aculeata*. Seven days later they were dissected out and examined. Two shells were empty and the other three contained a very small amount of living protoplasm. The former apparently were starved to death and the latter were almost dead of starvation.

Heteronema acus is captured but not injured by bladders. This green flagellate was used in 2 experiments.

Experiment 16.—Four bladders, 2 on each of 2 stems were inoculated with several specimens each. These bladders soon died and disintegrated but one of them still contained active specimens 3 days later.

Experiment 18.—Three stems bearing 7, 4 and 3 bladders respectively were placed in a culture containing *H. acus*. Twenty-four hours later specimens had been captured by 2 bladders on the first stem, 3 on the second and 3 on the third. Daily examinations were made for 5 days. During this time the flagellates retained their normal activities but apparently did not increase in numbers.

Phacus longicaudus is not injured by bladders and may multiply in bladders.

Experiment 28.—Twelve bladders on three stems were inoculated with from one to 7 specimens each of *P. longicaudus*. These specimens retained their normal appearance and activities. At the end of 7 days an increase in the number of specimens was noted in at least 5 bladders; these contained 6, 7, 8, 11, and 25 specimens each.

Stentor polymorphus is captured and killed by bladders.

Experiment M. 1.—One stem bearing 8 large and many smaller bladders was placed in a culture containing many specimens of *S. polymorphus*. Twenty-two hours later one bladder had captured 2 and another bladder one specimen. Two of the stentors were still alive with cilia moving but were abnormal in shape, the third had become spherical and quiescent.

Experiment M. 2.—Seven detached bladders were inoculated with stentors; 2 with one, 3 with 3, one with 4, and one with 6 specimens. Eighteen hours later the stentors in 3 of these bladders (7 stentors in all) had died and disintegrated; those in 2 bladders (5 specimens) were still active; and those in 2 bladders (9 specimens) were not active and abnormal in shape. One stentor remained active for 42 hours but all were dead and had disintegrated by the end of 66 hours.

Stylonychia pustulata is captured and killed by bladders.

Experiment M. 5.—Twelve large detached bladders were placed in a culture containing many specimens. Four hours later 5 had captured 1, 2, 2, 3 and 4 specimens respectively. These had all disappeared 20 hours later.

Experiment M. 3.—Eleven large detached bladders were inoculated each with a few specimens. Some of these were still alive and active 3 hours later. All but 2 specimens in one of the bladders had disappeared 20 hours later.

Experiment M. 4.—Eleven large detached bladders were inoculated with from one to 10 specimens each. Four hours later active specimens were noted in only 5 bladders, and 20 hours later only one specimen was found in one of them.

Colpidium colpoda is captured and may live from 7 to 24 hours or more. In seven experiments the culture used contained not only paramecia or stylonychias but also *C. colpoda*. The reactions of the latter were incidentally recorded.

Experiment M. 3.—Specimens of Colpidium were still alive in 8 of 11 detached bladders that had been inoculated 23 hours previously.

Experiment M. 4.—Specimens were alive and active in all cases 24 hours after inoculated into 11 detached bladders.

Experiment M. 7.—Colpidia were captured by all of 19 detached bladders but had disappeared in all but 2 seven hours later.

Experiment M. 11.—Colpidia were inoculated into 24 attached bladders (6 on each of 4 stems) that had previously been irrigated. They were active in at least 8 of these bladders 23 hours later.

Experiment M. 8.—Colpidia lived at least 48 hours in liquid drawn from 15 bladders and kept in a moist chamber in a hollow ground slide.

Experiment M. 10.—Colpidia lived at least 44 hours in liquid drawn from 15 bladders that had recently killed paramecia.

Chilomonas paramecium is captured by bladders. The medium used in Experiments 10, 24, and 25 contained many specimens of *C. paramecium* as well as paramecia, and it was noted that many of both the attached and detached bladders captured them. In some cases they may have entered at the same time paramecia were taken in but they were also present in bladders that had not captured paramecia.

Material containing intestinal flagellates of tadpoles quickly kills bladders. Four attempts were made (Experiments 3, 14, 21 and 22) to determine whether *Trichomonas augusta*, *Hexamitus batrachorum*, and *Euglenamorpha hegneri* from the intestine of tadpoles would be captured by and live in the bladders. Bladders were immersed in diluted intestinal contents containing these flagellates, and both attached and detached bladders were inoculated with them, but the bladders were quickly killed hence no definite results were obtained.

Nematodes and rotifers are captured by and live in bladders for many hours.

Experiment 4.—Several stems were immersed in a culture containing both euglenas and free-living nematodes; two days later 7 of the 13 bladders contained from 1 to 4 or more nematodes most of which were active.

Active rotifers were found in many of the bladders recently brought into the laboratory. These remained active for several days indicating that they are able to withstand conditions within the bladders for a considerable period.

Summary and Conclusions.

Summary. 1. The bladders (utricles) of *Utricularia vulgaris* v. *americana* Gray capture large numbers of small free-swimming aquatic organisms; those on parts of a plant 220 cm. long contained approximately 150,000 entomostraca and many other organisms.

2. Living protozoa of various kinds were found in the bladders; these were principally euglenas, but a few other flagellates, minute ciliates, and amœbæ were encountered.

3. Animals do not force their way into the bladders as hitherto supposed but are captured as follows: the normal bladder has compressed side walls; organisms enter the vestibule and by their movements stimulate the valve to open; water rushes in as the side walls expand and sucks in with it the stimulating organism; then the valve closes and the organism is unable to escape.

4. When a bladder expands its contents are increased approximately 88 per cent. by the outside medium which is sucked in with the prey.

5. Bladders expand when the wall is punctured and when the mouth opening is laterally or antero-posteriorly compressed.

6. Expanded bladders recover their compressed condition and are "set" for another capture in about 20 minutes. How 88 per cent. of their contents pass out during the period of compression is not known.

7. Euglenas occur frequently in bladders in nature but plants loose their infection if kept for many months in the laboratory. Free-living euglenas are captured by bladders and live and multiply within. They are not injured in bladders that have recently captured and killed paramecia. The euglenas that inhabit bladders in nature are probably captured free-living species and not species peculiar to the utricularia plant.

8. Paramecia are captured by bladders that are either attached to or detached from the plant; about 30 per cent. of bladders immersed in paramecium cultures succeeded in capturing one or more specimens within an hour. The number of paramecia captured by the bladders depends upon the culture medium only in so far as this affects the rate of swimming of the organisms and hence their chances of coming in contact with the valve of the bladders. Paramecia die within the bladders in an average period of about 75 minutes; some of them lived for many hours and some that were introduced into bladders with a fine pipette lived for 17 days. Paramecia die usually within several hours in bladders previously thoroughly cleaned out by irrigation.

Paramecia usually break up into granules within several hours after capture. Paramecia are not killed in old dead bladders nor in mature bladders killed by heat.

9. Specimens of the shelled rhizopod, *Centropyxis aculeata*, may be captured by bladders; they are not directly killed but die slowly of starvation.

10. The green flagellate, *Heteronema acus*, is captured but not injured by the bladders. *Phacus longicaudus*, is captured by bladders but not injured; it may multiply within the bladders. Bladders capture *Chilomonas paramecium*.

11. The ciliate, *Stentor polymorphus*, is captured and killed by bladders as is also *Stylonychia pustulata*. *Colpidium colpoda* is captured but may live for a day or more.

12. Material from the intestine of tadpoles containing flagellates was introduced into bladders, but the bladders were quickly killed.

13. Nematodes and rotifers captured by bladders live for many hours within.

Conclusions.—The principal conclusions from these studies are, (1) organisms do not force their way into bladders but are captured by them; (2) the bladders do not select their prey but any organism small enough to enter the vestibule may be captured; (3) organisms as small as *Chilomonas paramecium* (24 microns in length) may be captured; (4) the protozoa found in the bladders are not intruders but captives and are probably all free-living species; and (5) some of the captured protozoa live successfully within (*Euglena*, *Heteronema*, *Phacus*), some slowly starve to death (*Centropyxis*) and others are more or less quickly killed and digested (*Paramecium*, *Stentor*, *Stylonychia*, *Colpidium*). The data presented indicate that the bladders secrete something that kills and digests paramecia but does not injure euglenas. The origin and character of this secretion and the changes in the plant during the compression and expansion of the bladders are problems for the plant physiologist to solve.

LITERATURE.

Brumpt, E.

'25 Capture des larves de culicidés par les plantes du genre Utricularia. Ann. Parasit., 3: 403–411.

Büsgen, M.

'88 Ueber die Art und Bedeutung des Thierfangs bei Utricularia vulgaris L. Berichte der Deutschen Botanischen Gesellschaft, Bd. 6: LV–LXIII.

Darwin, Charles.

'75 Insectivorous Plants.

Goebel, K.

'91 Morphologische und biologische studien. Ann. du Jardin Botanique de Buitenzorg, 9: 1–126.

Hegner, R. W.

'22 The Effects of Changes in Diet on the Incidence, Distribution and Numbers of Certain Intestinal Protozoa of Frog and Toad Tadpoles. Journ. Parasit., 9: 51–87.

Hegner, R. W.

'23 The Effects of Changes in Diet on the Incidence, Distribution and Numbers of Certain Intestinal Protozoa of Rats. Amer. Journ. Hyg., 3: 180–200.

Hegner, R. W.

'23 Observations and Experiments on Euglenoidina in the Digestive Tract of Frog and Toad Tadpoles. BIOL. BULL., 45: 162–180.

Langeron, M.

'25 Biologie et écologie des utriculaires dans leurs rapports avec la prophylaxie du paludisme. Ann. Parasit., 3: 412–415.

Luetzelburg, P. von.

'10 Beiträge zur Kenntnis der Utricularien. Flora, 100: 145–212.

Mueller, H. O.

'25 The Rotifer Group. Nat. Hist., 25: 225–232.

Treat, Mary.

'75 Plants that Eat Animals. The Gardener's Chronicle, N.S., 3: 303–304. Reprinted from the New York Tribune.

THE PROTOZOA OF THE PITCHER PLANT, *SARRACENIA PURPUREA*.[1]

R. W. HEGNER.

Investigations of protozoa within the bladders of utricularia plants led the writer (Hegner, 1926) to examine the literature on other insectivorous plants and to carry out the experiments described below. The general problem under attack is that of host-parasite relationships and the specific problem is that of the relations between intestinal protozoa and their environment. Primitive conditions of digestion might be expected in insectivorous plants and this idea is responsible for the present investigation. Whether or not the liquid in the pitchers of the pitcher plants contains digestive enzymes secreted by the plant, which digest insects and other animals that enter the pitcher, is a problem that has been carefully studied by many investigators for many years. On several occasions when the problem seemed to be solved, a new factor was suggested that made it necessary to carry on further investigations. Among the most recent papers is that of Hepburn (1922) who concludes "on both morphological and bio-chemical grounds, that the pitchers of most, if not all, species of pitcher plants are designed for the capture and digestion of prey as a means of nutrition for the plant" (page 776). Among the species studied by Hepburn, St. John and Jones (1920) was *Sarracenia purpurea* and proteolytic enzymes (proteases) were found in the pitcher liquid of this species. Many types of experiments have been used to determine the character of the pitcher liquid but no one seems to have employed protozoa as indicators.

That living protozoa occur in the pitcher liquid of various species of pitcher plants has been mentioned by several investigators but only one worker (van Oye, 1921) has made a careful study of the species. In the pitcher liquid of *Nepenthes melam-*

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phora, van Oye noted 7 species of rhizopods, all shelled except 2 species of amœbæ. He identified these as *Centropyxis aculeata*, *Difflugia constricta*, *Lesquereusia epistomium*, *Arcella vulgaris*, *Cochliopodium bilimbosum*, *Amœba guttula*, and *Amœba nepenthesi*. *Amœba nepenthesi* is a new species named by van Oye. It is very small, with short, blunt pseudopodia and a body more or less egg-shaped. Cysts of it were found which soon opened when placed in a drop of water or in liquid from another plant, and liberated the amœba within.

Protozoa are present in the pitcher liquid of *Sarracenia purpurea* in nature. My examinations give only a general idea of the protozoan fauna of the pitchers. Several pitchers were examined from plants in the greenhouse of the university and ten pitchers were examined from plants obtained at Salisbury Cove, Maine. Organisms were looked for first in a sample of the liquid with a binocular microscope; then all of the liquid was centrifuged and the contents at the bottom of the centrifuge tube was studied with a compound microscope; and finally the debris at the bottom of the pitcher was examined. No attempt was made to identify definitely the various organisms encountered. Amœbæ, flagellates and small ciliates were found in the pitchers from Baltimore. Ten pitchers from Maine were taken from different plants growing in separate localities. They contained the following organisms.

1. Young pitcher containing 9 cc. of milky liquid. Protozoa, few small flagellates resembling *Cercomonas crassicauda;* insects, many ants, one house fly (?); insect larvæ, living, many; mites, one; rotifers, few.
2. Young pitcher containing 11 cc. of clear liquid. Protozoa, few cercomonas-like flagellates; insects, many, badly broken up; insect larvæ, living, several.
3. Mature pitcher containing 15 cc. of clear liquid. Protozoa, few cercomonas-like flagellates, several ridged flagellates, several radiosa-like amœbæ; insects, many, badly decayed; insect larvæ, several; mites, one; rotifers, few; nematodes, many.
4. Mature pitcher containing 7 cc. of milky liquid. Protozoa, many cercomonas-like flagellates; insects, many ants, one

house fly (?), one spider, one lace-wing fly; insect larvæ, living, many; mites, few; rotifers, many.

5. Mature pitcher containing 8 cc. of cloudy liquid. Protozoa, several varieties of cercomonas-like flagellates, many ridged flagellates, many amœbulæ, several limax amœbæ, several actimospherium-like rhizopods, many prorodon-like ciliates and many holosticha-like ciliates; insects, few, badly decayed; insect larvæ, living, many; rotifers, many; nematodes, many; entomostraca, few.
6. Mature pitcher containing 12 cc. of clear liquid. Protozoa, several cercomonas-like flagellates, many ridged flagellates, one amœbula; insects, few, badly decayed; insect larvæ, one; rotifers, many; entomostraca, one.
7. Young pitcher containing 17 cc. of clear liquid. Protozoa, many cercomonas-like flagellates; insects, few; insect larvæ, many; mites, few; rotifers, few.
8. Young pitcher containing 25 cc. of milky liquid. Protozoa, few bodo-like flagellates, few amœbulæ, many holosticha-like ciliates; insects, few; insect larvæ, many; mites, few; rotifers, few.
9. Young pitcher containing 19 cc. of clear liquid. Protozoa, many amœbulæ, many bodo-like flagellates; insects, many; insect larvæ, many.
10. Young pitcher containing 17 cc. of clear liquid. Protozoa, many cercomonas-like flagellates, few mastigamœba-like flagellates, few bodo-like flagellates; insects, few; insect larvæ, few; mites, one; rotifers, few.

Protozoa were present, as noted, in all ten pitchers and all three classes of free-living protozoa were represented by several species. The liquid from many other pitchers was examined in the experiments described below and similar protozoa were found in most of them and probably would have been found in all if a more careful search had been made. The most common living organisms encountered in the pitchers were insect larvæ, mites and rotifers. Nematodes and entomostraca were found in a few of the pitchers, and tardigradas in one.

How does the pitcher liquor become populated with protozoa? So far as I know no experimental data on this point exist. The

protozoa found living in the pitchers may be of two types, (1) free-living species and (2) species adapted for life in the pitcher liquid and restricted to this habitat. The pitchers are visited by many insects which are drowned in the liquid and these no doubt are frequently soiled with material containing cysts or even living trophozoites of free-living protozoa. This seems to be a simple avenue for the entrance of free-living species. Species that may be peculiar to the pitchers may similarly be transferred from one pitcher to another by the insects that live in the pitcher plant liquid and which fly from pitcher to pitcher to lay their eggs.

Experimental Studies on Free-living Protozoa in Pitcher Liquid.—Paramecia were the principal protozoa used in these experiments; Colpoda and Chilomonas were present in some of the paramecium cultures. Four types of experiments were performed; (1) paramecia were introduced into pitcher liquid in hollow ground slides, (2) into open pitchers in the laboratory, (3) into unopened pitchers in the laboratory and (4) into open pitchers in the field.

1. Paramecia in pitcher liquid in hollow ground slides: In three experiments liquid from 24 pitchers on plants brought into the laboratory was used. Five drops of liquid from each pitcher were placed in one hollow ground cavity of a slide and one drop of culture medium containing paramecia was added; five drops of culture medium were placed in the other cavity and one drop containing paramecia added. All the preparations were examined at frequent intervals during the first two days but after that daily for from 5 to 7 days. In every preparation the paramecia lived as well in the pitcher liquid as in the culture medium, and although no exact count was made, it was evident that multiplication occurred in at least 8 of the samples of pitcher liquid.

2. Paramecia in open pitchers in the laboratory: Paramecia were introduced into the liquid in 22 pitchers on four plants in the laboratory. The liquid in these pitchers was present when the plants were brought in. Eight of the pitchers were old, 8 mature, and 6 young. Samples were taken from these pitchers daily during the succeeding 5 days. Paramecia were recovered

from 15 of the 22 pitchers during this period. They may have been present in all, since it was discovered later that the paramecia tend to congregate at the bottom of the pitcher where the liquid is rich in organic debris; whereas the samples were taken from the liquid near the top. There was an evident increase in the number of paramecia present in at least 3 of the pitchers. Colpoda was recovered from 11 pitchers and apparently had multiplied in 4 of these.

3. Paramecium in unopened pitchers in the laboratory: Several cc. of culture medium containing paramecia were introduced into each of 4 unopened pitchers and the lips of the pitchers fastened together to prevent the entrance of insects. Samples were examined from these daily for 12 days. In 2 of the pitchers paramecium, and also colpoda and chilomonas, were more abundant at the end of the experiment than at the beginning; in the third pitcher paramecium was present in about the same numbers as at first; colpoda and chilomonas had increased; and in the fourth pitcher paramecia were present on the tenth day but absent on the eleventh and twelfth days; colpoda decreased in numbers but chilomonas had increased.

4. Paramecium in open pitchers in the field: In order to avoid the possibility that changes might occur in the pitcher liquid when plants are brought into the laboratory, paramecia were introduced into ten pitchers borne by 5 plants in the field. These were labelled and examined on the following day and 2 days later. Paramecia were present in only 2 samples taken after 24 hours, colpoda in 7 and chilomonas in 7. On the second day the pitchers were brought into the laboratory, except one which had lost its label and could not be identified, and paramecia were recovered in all 9 of them. Apparently no decrease in numbers had occurred in the 48 hours of the experiment and increases seemed probable in several pitchers. The reason paramecia were found on the first examination in only 2 pitchers is that the organisms had congregated near the bottom of the pitcher where food conditions were favorable and, therefore, could not be recovered from the samples which were taken near the surface. Colpoda and chilomonas were recovered also from all 9 pitchers with no apparent decrease in numbers.

It was a surprise to find that paramecia are able to withstand the digestive enzymes in the pitcher liquid. There may still be some question as to whether these enzymes are produced by the plant or by bacteria, but their presence is not generally disputed by investigators. According to Dr. Edgar T. Wherry (in letter) the pitcher liquid varies widely in reaction when the hydrogen-ion concentration is measured so that the paramecia in my experiments were probably living in solutions that differed considerably in this respect. That paramecia are able to withstand conditions within the digestive tract of frogs has been shown by Cleveland (MS). Rich cultures of paramecia were injected into the rectum of living frogs; in some cases one or less per cent. of them became encysted within usually from one to 2 hours; the rest were killed. In one instance encystment occurred within 30 minutes.

So far as the problem is concerned that the experiments described above were designed to solve, the conclusions seem justified that, (1) paramecia are able to live within the liquid either outside or within the pitchers of various ages borne by vigorous plants for an indefinite period without injury; (2) paramecia may multiply in the pitcher liquid; (3) colpoda and chilomonas are similar to paramecia in these respects; (4) the protozoa that occur in nature in the pitcher liquid are probably free-living species carried there by flies although some of them may be species restricted to this particular environment.

LITERATURE CITED.

Hegner, R. W.

'26 The Interrelations of Protozoa and the Utricles of Utricularia. BIOL. BULL. (In press.)

Hepburn, J. S.

'22 Biochemical Observations on Certain Insectivorous Plants. Journ. Franklin Institute, 194: 771–781.

Hepburn, J. S., St. Johns, E. G., and Jones, F. M.

'20 The Absorption of Nutrients and Allied Phenomena in the Pitchers of the Sarraceniaceæ. Journ. Franklin Institute, 189: 147–184.

van Oye, P.

'21 Zur Biologie du Kanne von Nepenthes melamphora. Biol. Centrlb., 41: 529–534.